Thesis for doctoral degree (Ph.D.)
2013

Famine in Nigeria and vitamin D in Sweden: two early life exposures and their relation to cardiovascular risk in adulthood

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“Inte halvvägs ut till Farsta, inte bland alla tallarna där ute, inte halvvägs ut till Farsta”

*- Thåström**

This is mainly an ebook

Published by **Karolinska Institutet**
Stockholm, Sweden

ISBN 978-91-7549-302-2

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ABSTRACT

Early life environment has in previous research been linked to risk of disease in adulthood. This thesis investigated three types of early life exposures and their potential associations with adult life cardiovascular risk.

It has been proposed that early life malnutrition underpins the ongoing epidemic of lifestyle-related diseases in Sub-Saharan Africa. We examined the association between exposure to the Biafra famine (1968-1970) and cardiovascular risk in 1,339 Nigerians. Individuals exposed to famine in fetal-infant life had higher blood pressure, plasma glucose and BMI compared to individuals born after the famine. Malnutrition in early life may contribute to the burden of lifestyle-related disease in Sub-Saharan Africa (Paper I).

Vitamin D deficiency is prevalent among women of childbearing age worldwide. Adult vitamin D deficiency has been linked to increased risk of cardiovascular disease, impaired glucose tolerance and obesity. We aimed to assess whether vitamin D status at birth is related to cardiovascular risk in adulthood.

In paper II, neonatal vitamin D concentrations from stored blood samples were measured and cardiovascular risk markers assessed in 275 individuals aged 35 years born either in the end of the summer or in the end of the winter. We found no associations between low neonatal vitamin D status and cardiovascular risk at 35 years of age. However, men and women in the highest neonatal vitamin D quintile were at higher risk of being overweight (Paper II).

The prime determinant of vitamin D status is exposure to sunlight. Month of birth is a proxy for a number of seasonally dependent environmental exposures including nutrition, infections, lifestyle factors – and vitamin D. At high latitudes, vitamin D levels in populations are lower in the winter compared to the summer due to scarce sunlight exposure. In the Swedish population aged 30 or above (>6 million individuals), followed from 1991 during 20 years, individuals born during autumn months lived longer than those born during spring months. The association between month of birth and mortality was particularly pronounced in the age-span 50 to 80 years and not significant before 50 years (Paper III). In the age-span 50 to 80 years, cardiovascular mortality was increased among spring-born compared to autumn-born. (Paper IV)

Although individuals born in Sweden during the spring had an increased risk of cardiovascular mortality in ages 50 to 80 years (paper IV), the effect sizes were small. The lack of an association between low neonatal vitamin D status and adult cardiovascular risk in paper II indicate that vitamin D levels at birth may not be of sizeable importance to adult life cardiovascular health.

PAPERS

This thesis is based on the following papers. The papers will be referred to by their number: I-IV.

I. **Hult M*, Tornhammar P*, Ueda P***, Chima C, Ozumba B, Edstedt Bonamy A-K, Norman M. (2010) Hypertension, Diabetes and Overweight: Looming Legacies of the Biafran Famine. PLoS ONE 5(10): e13582. doi:10.1371/journal.pone.0013582

* shared lead author position

II. **Tornhammar P*, Ueda P*, Hult M***, Simila H, Eyles D, Norman M. Season of birth, neonatal vitamin D and cardiovascular risk at 35 years of age – a cohort study from Sweden
Submitted

* shared lead author position

III. Ueda P, Edstedt Bonamy A-K, Granath F, Cnattingius S (2013) Month of Birth and Mortality in Sweden: A Nation-Wide Population-Based Cohort Study. PLoS ONE 8(2): e56425. doi:10.1371/journal.pone.0056425

IV. Ueda P, Edstedt Bonamy A-K, Granath F, Cnattingius S Month of birth and cause-specific mortality between 50 and 80 years: a population-based longitudinal cohort study in Sweden
Submitted.

INTRODUCTION & BACKGROUND

Rural Enugu, Nigeria

INTRODUCTION

Epidemiological evidence suggests that the risk of lifestyle-related diseases emerging in adult age is determined not only by genetics and lifestyle but also by factors acting in pre- and early postnatal life. The "developmental origins of health and disease" (DOHaD) hypothesis has yielded vast amounts of literature evaluating associations between early-life exposures - in particular related to nutrition - and later risk of disease.

This thesis aims to shed light on three different early-life exposures in two different parts of the world.

First, the role of severe malnutrition in fetal and infant life, for later cardiovascular and metabolic risk is assessed in a Nigerian cohort based on the tragic circumstances during the Biafra famine in the late 1960s. This is the first study on the topic conducted in sub-Saharan Africa; a part of the world with an ongoing maternal-infant undernutrition and a rapidly increasing prevalence of lifestyle-related disease. (Paper I)

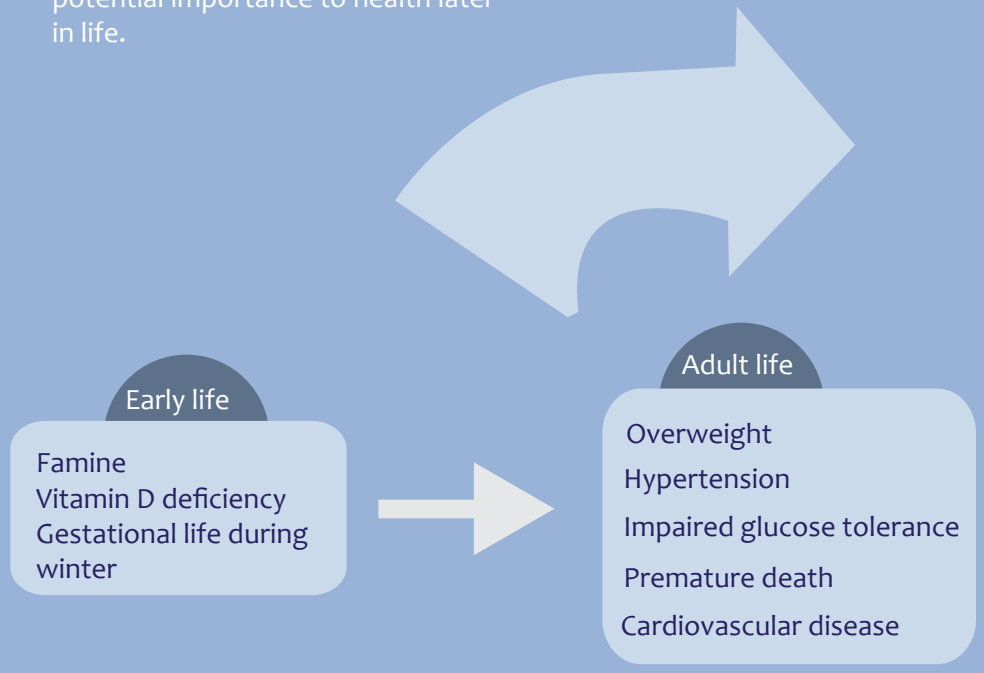
Second, the relation between vitamin D levels at the time of birth and cardiovascular risk at the age of 35 is assessed in a group of Swedes born in either the end of the winter or in the end of the summer. (Paper II)

Neonatal vitamin D status depends on maternal serum concentrations which in turn are largely dependent on exposure to sunlight. Vitamin D status has in adult cohorts been shown to correlate to risk of the metabolic syndrome and cardiovascular mortality. It has been suggested that vitamin D deficiency in early life predisposes the fetus for adult life cardiovascular disease. Sweden with its large differences in sunlight hours between the seasons is an apt region to assess this hypothesis.

Third, month of birth is a proxy for maternal sun exposure and subsequent vitamin D levels during pregnancy as well as other seasonally dependent influences. The relation between month of birth and mortality in different ages and from different causes is therefore examined using data from the whole Swedish population in 1991 which is followed during 20 years. (Paper III&IV)

Previous research shows that exposures in early life is associated with cardiovascular risk in adult life.

This thesis aimed to investigate three types of early life exposures of potential importance to health later in life.



	RATIONALE	HYPOTHESIS	METHODS
THE BIAFRA COHORT	Ongoing fetal malnutrition and a growing epidemic of lifestyle-related diseases in Sub-Saharan Africa	Early life exposure to the Biafra famine is associated with increased blood pressure, plasma glucose and BMI	Cohort study based on exposure to the Biafra famine during 1968-1970. Study participants born before, during or after the years of famine
NEONATAL VITAMIN D & CARDIOVASCULAR RISK	Vitamin D deficiency is prevalent among pregnant women and adult vitamin D deficiency is linked to risk of lifestyle-related disease	Low vitamin D at birth is associated with increased cardiovascular risk at 35 years of age in Sweden	Cardiovascular risk markers assessed in 284 individuals 35 years of age in Sweden. Their vitamin D at birth was measured in stored neonatal blood samples
MONTH OF BIRTH AND MORTALITY	Month of birth - a proxy for a variety of seasonally dependent exposures including vitamin D- is linked to adult age mortality	Month of birth is associated with adult age mortality, in particular cardiovascular mortality, in Sweden	Longitudinal study including the whole Swedish population in 1991, followed for 20 years until death, emigration or end of follow-up

BACKGROUND

The burden of lifestyle-related diseases is growing globally

Lifestyle-related disease refers in this thesis primarily to cardiovascular disease and type 2 diabetes as well as its risk conditions hypertension, impaired glucose tolerance and overweight/obesity. Although the definitions vary, these groups of diseases are often referred to as the metabolic syndrome, which also include measures of blood lipids and inflammation.[1]

The burden of lifestyle-related disease is increasing globally and poses a tremendous challenge for health care systems and patients. Globally, 366 million individuals were estimated to have diabetes in 2011; a number that is projected to reach 552 million by 2030.[2] Cardiovascular disease causes 3 out of 10 deaths worldwide constituting the most common cause of death.[3] Previously regarded as a problem of high-income countries, lifestyle-related diseases are now increasing at an unprecedented rate in low and middle income countries. For example, India is today the country with the highest number of diabetes patients in the world and over 80% of cardiovascular deaths worldwide occur in low- and middle income countries.[3]

Once developed, lifestyle-related diseases often require lifelong medication and management. As the name implies, this group of diseases are to a large extent preventable by modifying lifestyle-related risk factors such as lack of exercise, smoking and poor diet. However, research also highlights the importance of early life environment for disease risk later in life.

Given the pressing burden of lifestyle-related disease globally, especially in low income countries where health care resources are scarce, it is of utmost importance to identify risk factors – starting already from prenatal life - and outline efficient public health measures to curb the growing epidemic.

Developmental origins of health and disease

A significant body of evidence supports the theory that susceptibility for lifestyle related disease in adult age - including cardiovascular disease, type 2 diabetes mellitus, obesity and their preconditions - is influenced by exposures in early life. Since Barker and Osmond launched the model of "developmental origins of health and disease" (DOHaD), by showing that low birth weight was associated with risk of cardiovascular events in England and Wales[4], the concept has been extensively investigated, yielding a number of complementary hypotheses.

The thrifty-phenotype hypothesis – a theory aiming to explain DOHaD - proposed that early life exposure to nutritional paucity regulates genes to operate in a manner suited for a nutritionally suboptimal environment. These modifications of the phenotype may improve chances of short-term survival and reproduction but may entail deleterious effects on long term health.[5] The DOHaD and the thrifty phenotype hypothesis were further expanded by Hanson, Gluckman and colleagues[6] who introduced three interacting terms to explain the concept: suboptimal environment, predictive-adaptive response and distance of fit. The predictive-adaptive response is the change in the developmental course of the fetus or the infant induced by the early environment to optimize fitness in the predicted environment in later life. The distance of fit is how well the predictions of the environment during the critical periods of developmental plasticity match the encountered environment in adult age. The concept proposes not only alterations in the phenotype but also other developmental adaptations such as a reallocation of fetal circulation to essential organs at the expense of other less important systems to optimize short term survival. DOHaD is supported by a number of studies in both humans and animals, including exposures such as malnutrition and intrauterine growth restriction.

The Dutch famine cohort

The Dutch famine occurred during the winter of 1944 as a result of the Second World War German occupation. During a four-month period, the daily rations were between 400 and 800 calories. Individuals conceived and delivered in connection to this period have been studied for assessment of the relation between early life malnutrition and adult health outcomes. The Dutch famine cohort constitutes of 2414 participants categorized into individuals exposed to famine in early, mid or late gestation, depending on the timing of the famine in relation to their birth date.

Individuals who were born before or conceived after the famine constituted the control group. [7]

Individuals exposed to famine in late or mid gestation were of smaller body size and weighed significantly less at birth than babies unexposed to the famine.[7] Exposure to famine in late gestation was associated with impaired glucose tolerance. This association remained even after adjusting for birth weight.[8] Further, early gestation exposure to famine was not associated with birth weight but with a threefold increase in risk of coronary heart disease[9], levels of plasma fibrinogen[10], obesity[11] and a more atherogenic lipid profile[12].

Although a link was found between low birth weight and high blood pressure, exposure to famine during gestation was not associated with blood pressure at ages around 50.[13] However, in another study with follow-up at 59 years of age, individuals prenatally exposed to at least 10 weeks of famine had increased blood pressure and were at increased risk of hypertension.[14]

Other famine studies

Exposure to famine in different ages and its relation to long term health have also been studied among survivors of the Leningrad (St Petersburg) siege (1941-1944) during the Second World War. When comparing individuals exposed to malnutrition in utero (n=169) with those exposed in infancy (n= 192) there were no significant differences in glucose tolerance, blood pressure, lipid concentration or coagulation factors at the age of 52 to 53. The group exposed in utero had endothelial dysfunction shown by higher concentrations of von Willenbrand factor. [15] Further, in this group, obesity was a stronger predictor of increased blood pressure than it was among the group exposed in infancy. The study also included a control group born during the same time period but in regions unaffected by the siege. Compared to the two exposed groups, the control group differed with respect to several of the outcomes. These differences however, were considered to relate to different selection criteria.[15] It has been hypothesized that the lack of an effect of fetal malnutrition on most of the cardiovascular and metabolic risk markers in the Leningrad study is due to the region being relatively scarce in nutrition both before and after the famine. Thus, the Leningrad setting did not introduce a pre- and postnatal nutritional mismatch and potential catch-up growth as seen in the Dutch famine studies.[15] Of note are also the relatively small sample sizes of the Leningrad studies.

In China, individuals severely exposed to the Chinese famine (1959-1961) during fetal life were at increased risk of hyperglycemia[16] and overweight[17] compared to unexposed individuals.

Low birth weight as a proxy for early life stress and malnutrition

The lion share of the literature on DOHaD has investigated effects of early life stress- indicated by impaired fetal growth or exposure to malnutrition – on adult health. Although controversial due to risk of misclassification and confounding, low birth weight is a widely studied proxy for intrauterine stress. Low birth weight has in a large number of studies been linked to risk conditions for lifestyle-related disease such as dyslipidemia, impaired glucose tolerance, hypertension, vascular endothelial dysfunction and overweight.[18]

Exposures during infancy and childhood is potentially also of importance to long term health. Seemingly, the risk of disease attributed to birth weight is larger for children born smaller and who later in life grow obese. An accelerated weight gain exhibited by low birth weight infants relative to normal weight infants, has been proposed to have adverse effects on long term health and is associated with risk of obesity in adulthood.[19, 20]

Early life-factors with life-course interpretations?

There are a number of important arguments against the notion that early life nutrition constitutes an important modifiable risk factor for adult life disease. These are particularly articulated for studies using low birth weight as a proxy for fetal stress and malnutrition. First, confounding by socioeconomic status could potentially contribute to spurious associations between birth weight and adult cardiovascular disease.[21] Second, maternal pre-pregnancy hypertension or pregnancy-induced hypertension may also be associated with reduced fetal growth. A propensity to develop these conditions may be passed on to the offspring by the mother through shared genetic and lifestyle related factors.[21] Furthermore, twin studies indicate that the association between fetal growth and later cardiovascular disease is possibly attributable to genetic factors shared by the mother and the child[22].

As for famine studies, selective survival remains a concern. It is possible that infants that are genetically better prepared for nutritional hardships survive malnutrition in utero and reach adult age. These individuals may also be more susceptible to lifestyle-related diseases compared to

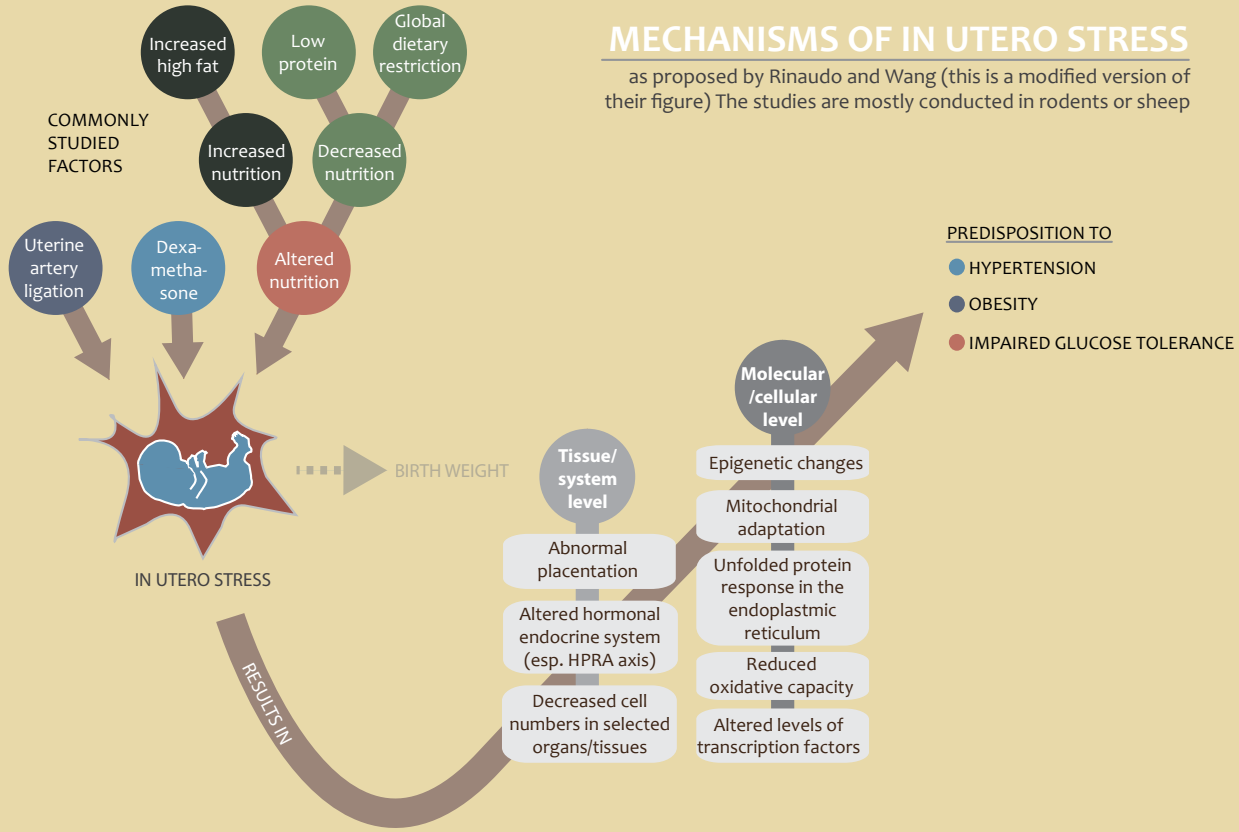
Early life nutrition

individuals who did not survive.

Another important point of criticism pertains to the public health relevance of the hypothesis. Even if early life factors would be proven to cause increased risk of lifestyle-related disease, is the magnitude of the effect large enough to justify attention and interventions by policy makers? Ecological data on trends in birth weight and cardiovascular disease from different parts of the world are, arguably, not convincing of a substantial effect of birth weight on the adult age disease burden. The increasing prevalence of lifestyle related diseases in developing countries has largely been attributed to adult age risk factors such as smoking, physical inactivity and diet.[21] A 1-kg increase in birth weight adjusted for gestational age has been associated with a fall of 1.6 mmHg in systolic blood pressure; certainly of importance for the prevalence of hypertension on population level. However, a 1kg difference in birth weight is huge; an equivalent of five or six times the difference in average birth weight between infants of smoking and non-smoking mothers.[21]

Suggested mechanisms

Several mechanisms explaining associations between in utero stress and adult health observed in human populations have been suggested based on studies on animals. Rinaudo and Wang[18], categorize them into:



- *Cellular responses to stress*, including epigenetic changes, mitochondrial dysfunction and oxidative stress.

- *Alterations in adult organ morphology or cell number*, e.g. adaptations to suboptimal environment by prioritizing the development of essential organs such as the brain at the expense of the development of less essential organs, for example by decreasing nephron mass in kidneys or beta-cell mass in pancreas.

-*Tissue or systemic responses*, e.g. alternations in the placenta or in the endocrine pathways such as the HPA-axis.

- *A combination of the above*

Is the increasing burden of lifestyle-related diseases in Sub-Saharan Africa underpinned by early life malnutrition?

The theory of a mismatch between a nutritionally sparse intrauterine environment and a postnatal life where nutrition is abound as predisposing for lifestyle-related disease is of particular interest in low- and middle income countries. In these parts of the world, the standard of living increases substantially within one generation. People are born in nutrient scarce rural areas and move to urban areas where they are exposed to various risk factors for lifestyle-related disease. It is thus proposed that the growing burden of lifestyle-related disease in developing economies worldwide could be underpinned by early life exposure to limited nutrition.[27, 28]

Although the definition of malnutrition also includes overnutrition, it generally refers to a state when the body does not get enough nutrients to ensure growth, maintenance and specific functions.[29] Sub-Saharan Africa is a part of the world with an unresolved situation of malnutrition and starvation. According to the WHO 27-51% of women of reproductive age are underweight in Africa[30] and famines are ongoing on the continent as results of political conflicts.

Concurrently, the prevalence of lifestyle-related disease is increasing rapidly, particularly in urban areas. It is projected that deaths from ischemic heart disease will increase twofold by 2030 and yearly

kill over 700 000 in Sub-Saharan Africa [31]. Similarly, the diabetes rates of the continent are soaring. In 2010, 12.1 million people were estimated to have diabetes; a number that is expected to increase to 23.9 million until 2030[32], when the African region is projected to have the largest proportional increase in adult diabetes in the world.[33] In the light of the paralleling epidemics of malnutrition and lifestyle-related disease in Sub-Saharan Africa, it is important to investigate the link between early life malnutrition and later cardiovascular and metabolic health in this setting.

Month of birth, early life exposures and health outcomes

The findings of in utero stress, indicated by birth weight and malnutrition, as an antecedent of adult disease has spurred interest in elucidating other early life factors - both nutritional and non-nutritional – of importance to adult health.

One broad indicator for early-life events is month of birth. Environmental exposures in pre- and postnatal life possibly associated with month of birth include maternal and infant infections, temperature, nutrition, sunlight exposure and lifestyle factors. Although the findings are debated, risk of schizophrenia and multiple sclerosis[34] have been linked to month of birth. A number of studies have also reported that late-life health and life expectancy are influenced by factors associated with month of birth.

Cross-sectional studies from the northern hemisphere based on age at death have demonstrated that people born in the autumn (October-December) live longer than those born during spring (April-June). [35–38] This pattern was mirrored on the southern hemisphere, where spring-born live longer than those born during autumn.[36] The difference between the months of birth with the lowest and highest average age at death ranges between 3.6 months (Denmark)[36] to 9.6 months in Germany.[38] A study on a U.S. population showed that people born in autumn had a higher chance of surviving to the age of 100 compared to spring-born. Comparison was made with siblings and spouses thus controlling for unobserved shared childhood or adulthood environment and common genetic factors.[37] Month of birth-studies are ideally conducted with longitudinal data including information about birth date and death for the whole population at risk. [35] To date, there is only one study on the topic that has followed a population longitudinally. A closed cohort of more than 1,3 million Danes aged 50+ was followed for 30 years. Paralleling other observations, autumn born were shown to have a longer remaining post-50 life expectancy compared to spring born.[36]

In cross-sectional studies assessing death certificates in the U.S., the month of birth-effects on mortality were present in a diverse range of death causes, in particular cardiovascular disease and malignant neoplasms, but also suicide and infections. [35] In a study including subjects dying from cardiovascular causes in Germany between 1992 and 2007, men born in May lived 11.7 months shorter than men born in November. For women, the difference was 7.3 months. [38]

Month of birth and lifespan: hypotheses

Doblhammer and Vaupel[36] investigated a number of hypotheses possibly explaining the month of birth-related differences in mortality.

EPIGENETIC CHANGES

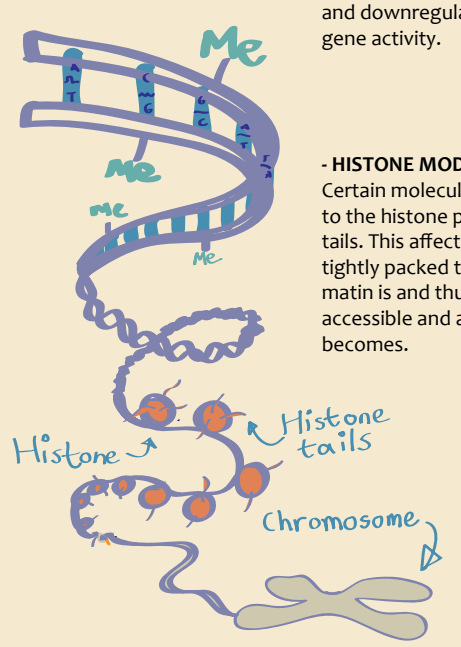
Recent research has highlighted epigenetics as "the link between genome and environment". Epigenetics is the modification of gene function without the sequence of the genes being changed. Epigenetic mechanisms regulating the expression of genes include modification of cytosine bases and conformational changes in chromatin, most commonly histone modification opening or closing the chromatin structure. These epigenetic changes result in the silencing or activation of gene transcription.

The Agouti mouse constitutes a famous example of how epigenetic changes induced in prenatal life can alter the adult phenotype. Mice supplied with folic acid in utero through maternal supply, develop a greyish fur that is not seen in controls. The altered phenotype has been attributed to DNA-methylation in genes regulating the color of the fur.[24]

There are also several examples of developmental plasticity, where organisms change structure or function dependent on early environmental input that indicate postnatal environment. The Daphnia develops a helmet formation on its body in response to early life environmental cues about risk of predation.[25] The locust, Locusta migratoria adjusts its wing shape and metabolic pathways in the larva stage depending on pheromone signals which are indicators of population density.[26]

Hypothesizing on an evolutionary perspective of DOHaD, Hanson and Gluckman posited that fetal reprogramming– occurring partly through epigenetic changes – induced by in utero exposures is a short term adaptation to the expected environment in order to maximize the survival for the individual. The "survival of the fittest individuals", on the other hand, creates long term adaptation to the environment for the species.[6] Epigenetics could also explain the transgenerational effects of inutero exposures as seen in both animals and humans.

TWO MAIN EPIGENETIC MECHANISMS
A modified version of a figure presented by Qiu J [36]



- DNA METHYLATION
Methyl groups are added to DNA-bases and downregulate gene activity.

- HISTONE MODIFICATION
Certain molecules attach to the histone proteins tails. This affects how tightly packed the chromatin is and thus how accessible and active it becomes.

Socioeconomic seasonality in birth rates:
Socioeconomic status is a life course risk factor for disease. Certain socioeconomic groups may be overrepresented in certain birth seasons. The differences in mortality would thus only reflect a socioeconomic seasonality in child birth.

Seasonal distribution of deaths:
There is a possibility that death peaks during certain parts of the year cause statistical artifacts of month of birth-related differences in adult age mortality.

For example, individuals born in May are always older than the fall-born when the increased mortality risk of winter strikes and they may therefore have a higher overall mortality.

Selective survival early in life:
Robust individuals more likely to survive seasonally dependent early life exposures may also be more susceptible to adult age disease, in particular lifestyle-related diseases.

Analyzing data from Australia, Denmark and the U.S., Doblhammer and Vaupel concluded that differences in lifespan by month of birth are most likely to stem from debilitation in utero or infancy due to seasonality in nutrition availability or infections.[36]

Nutrition and infections:
In the beginning of the 20th century, when the individuals included in the existing month of birth-studies were born, food supply, especially fruit and vegetables, fluctuated by season. The DOHaD hypothesis suggests that nutritional exposures in early life affect late life health outcomes. Furthermore, debilitation in utero or in post-natal life due to seasonal infections is also a relevant hypothesis supported by epidemiological data.

Vitamin D: a seasonally dependent nutritional exposure

Another seasonally dependent nutritional exposure is vitamin D. Exposure of skin to sunlight is the prime determinant for the production of the vitamin –clinically measured as the metabolite 25–hydroxyvitamin D (25(OH)D).[39]

The fetus depends entirely on maternal supply for its vitamin D-levels.[40] Depending on latitude, sunlight hours can differ substantially between seasons, and 25(OH)D levels in populations are lower in the winter compared to the summer.[41–43] It is thus likely that individuals born in higher latitudes are exposed to different levels of vitamin D – due to differences in maternal sun light exposure - in fetal and neonatal life depending on their month of birth.

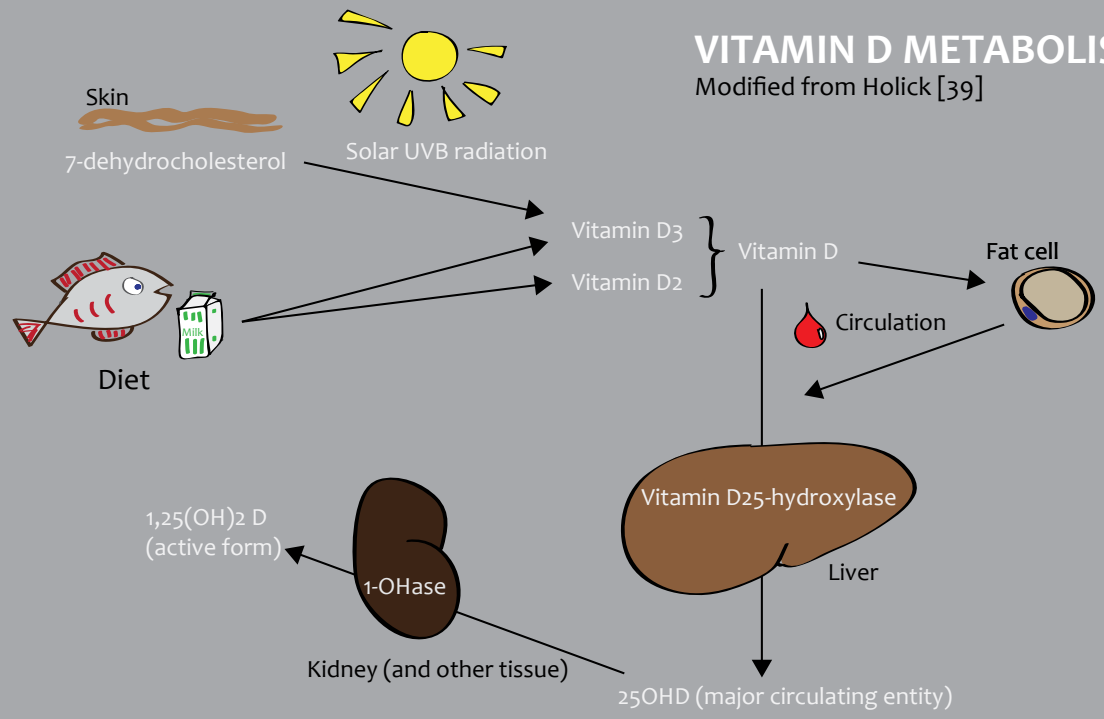
Vitamin D is primarily known for its importance in the calcium metabolism. Severe deficiency causes rickets and bone malformation. It has been shown, however, that the majority of the cells and tissues in the body have the vitamin D receptor and that many of them contain the enzymes (1-alpha-hydroxylase) converting circulating 25-hydroxyvitamin D to the active form 1,25-hydroxyvitamin D. This discovery, along with epidemiological clues, has opened up for research on the potential role of vitamin D in the risk of many diseases, including autoimmune diseases, infectious diseases, cancers and cardiovascular diseases.

Vitamin D and lifestyle-related disease

Numerous cross-sectional and prospective studies have linked low vitamin D-levels to cardiovascular risk conditions and the metabolic syndrome.[44, 45]

In a study from the U.S, it was shown that vitamin D-deficient men were at a more than twofold risk of myocardial infarction compared to vitamin D sufficient men, after controlling for a number of confounders including BMI, blood lipids and ethnicity.[46] In line with these findings were observations from Germany showing increased risk of all-cause as well as cardiovascular mortality for subjects with severe vitamin D deficiency compared to subjects with optimal vitamin D levels.[47] Studies also report associations between low vitamin D levels and increased risk of type 2 diabetes[48, 49], dyslipidaemia[50, 51], elevated blood pressure[52] and obesity[53, 54], although for some of the outcomes, results are partly conflicting[45].

Causality regarding the link between low vitamin D and lifestyle related diseases is debated. As vitamin D levels are influenced by life-style factors, including obesity, physical activity outdoors and fat fish

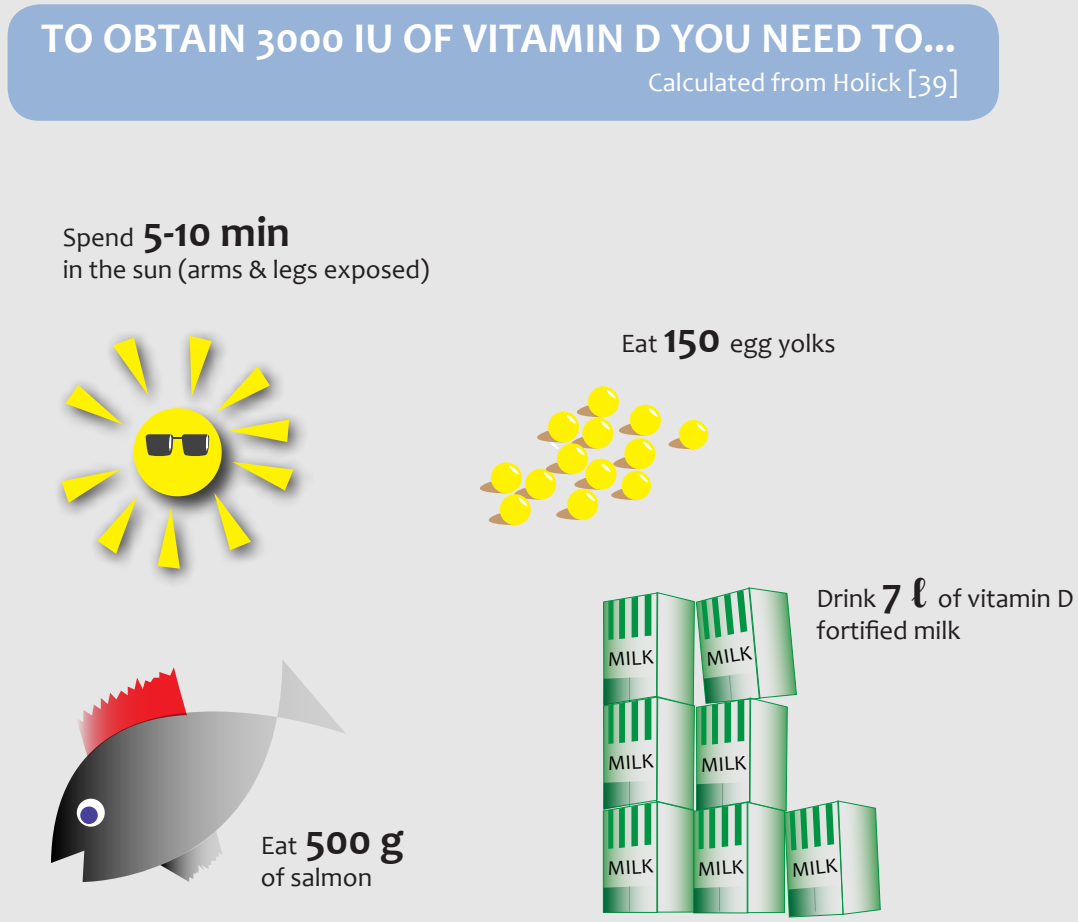


Exposure of skin to sunlight is the prime determinant for vitamin D-production. Ultraviolet radiation (UVB) penetrates the skin and acts on a cholesterol metabolite in epidermis which is subsequently hydroxylized in the liver to 25-hydroxyvitamin D (25(OH)D) – used to determine vitamin D status in patients. 25(OH)D can exist in two forms: 25(OH)D3 and 25(OH)D2; the latter can only be obtained from a number of dietary sources including fat fish and supplements. A second hydroxylation –largely occurring in the kidney but also in many other tissues- produces 1,25hydroxyvitamin D which is the biologically active form of vitamin D.

Recommended intake of vitamin D and the definitions of vitamin D deficiency and insufficiency are subject to debate. Recent literature defines deficiency as 25(OH)D <50 nmol/L and insufficiency as 25(OH) D<70nmol/L.[39] Risk factors for low vitamin D include factors associated with UVB exposure, i.e. season, latitude, skin pigment and sunscreen use as well as conditions affecting the bioavailability or metabolism such as obesity (sequestration of vitamin D in body fat) and malabsorption of fat.[38]

consumption[39], confounding remains a concern. Data from randomized controlled trials with vitamin D supplementation are limited and have partially, but not consistently, indicated improved outcomes for some cardiovascular risk factors including arterial hypertension. Meta-analyses of RCTs indicate modestly reduced all-cause mortality in vitamin D-supplemented subjects.[55]

Several biological mechanisms explaining how vitamin D could be of importance to cardiovascular and metabolic diseases have been proposed and supported with experimental data. The vitamin D receptor is present in vascular smooth muscle, endothelium, as well as in cardiomyocytes.[56, 57] Pancreatic beta-cells contain both the vitamin D receptor and 1-alpha-hydroxylase.[55, 58] Modifications of these struc-



tures affect the glucose and insulin levels in mice.[58] Vitamin D is also involved in the renin-angiotensin-aldosterone system[55, 59] regulating blood pressure.

Early-life vitamin D and lifestyle-related disease

It has been proposed that the prenatal period may constitute a critical window during which vitamin D deficiency predisposes the fetus for adult age cardiovascular and metabolic disease.[60–62]
In studies on rodents, maternal vitamin D-deficiency led to retarded cardiac development in the offspring[63] and to alterations in the kidney development of possible importance to later blood pressure[64]. Maternal vitamin D-deficiency in the third trimester in an Indian population was associated with increased fasting insulin resistance and reduced arm muscle area in the offspring at the age of 9.5 years.[65] In England, low vitamin D during the third trimester of pregnancy in mothers was linked to adiposity in their 6 year old children.[66] However, in studies with follow-up limited to childhood ages, no associations have been shown between early life vitamin D and a number of other cardiovascular and metabolic risk markers.[65, 67]

Given the high prevalence of vitamin D deficiency in women of childbearing age observed in different parts of the world [68–72], the link between early life vitamin D and adult cardiovascular health is of interest to study further. To date, there are no studies assessing this hypothesis with a follow-up time reaching beyond childhood.

Furthermore, Sweden is a country with large differences in sunlight hours between the seasons. It would thus be of interest to study the relation between month of birth – as a proxy for early life vitamin D – and adult life mortality on population level, in particular as it pertains to cardiovascular diseases.

ETHICS APPROVAL

The studies included in this thesis were approved by the regional ethics committee in Stockholm. Paper I was also approved by the Institutional Review Board at the University of Nigeria Teaching Hospital, Enugu.

Paper I: 2009/695-31/1
Paper II: 2010/739-31/3
Paper III&IV: 2011/19803

AIMS

The Biafra cohort (Paper I)

To examine the relation between exposure to the Biafra famine (1968-1970) in early life and risk of adult hypertension, diabetes and overweight in a population of 40 year old Nigerians.

Neonatal vitamin D and adult cardiovascular risk (Paper II)

To assess the link between season of birth, neonatal vitamin D status and cardiometabolic risk at the age of 35 years.

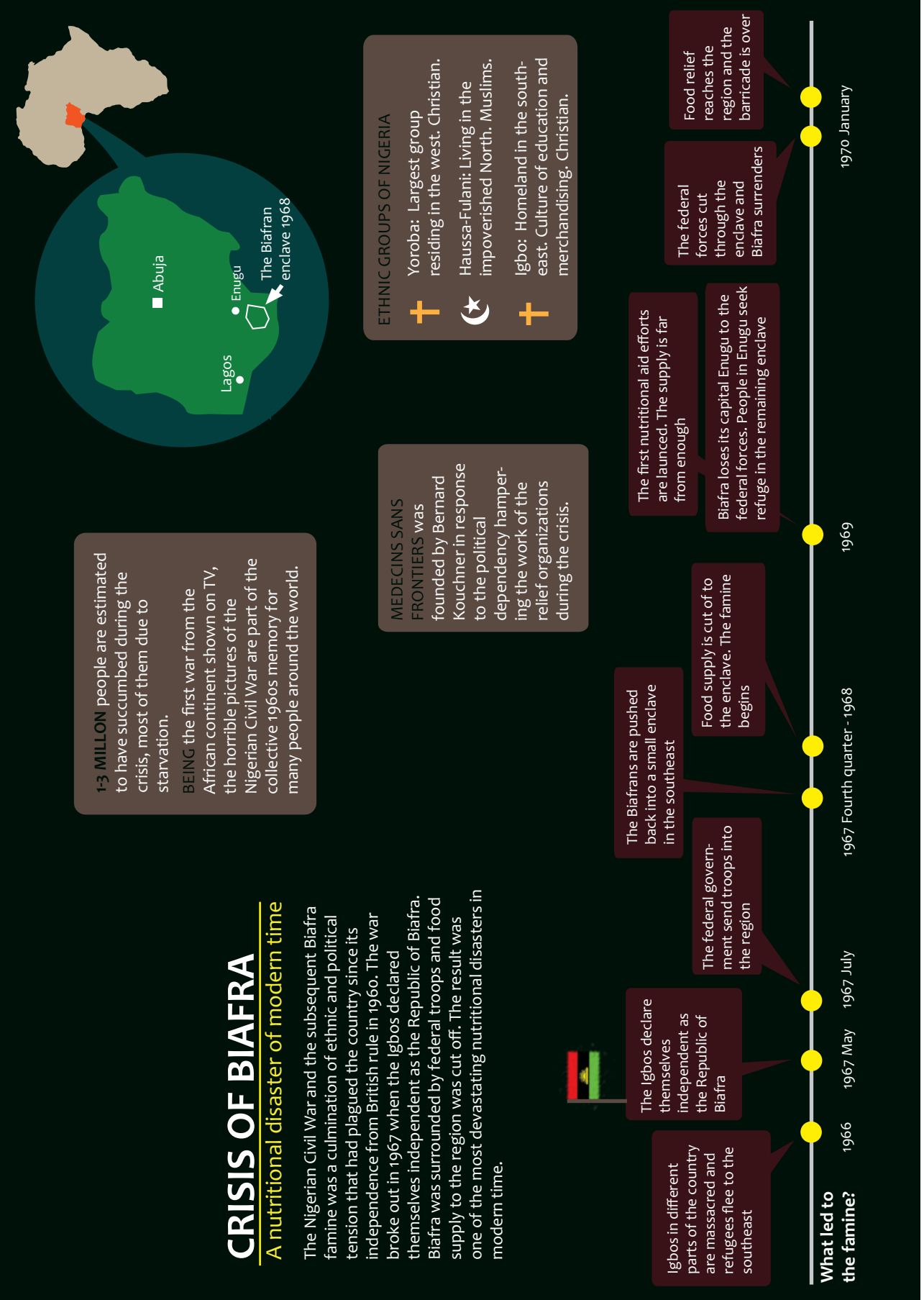
Month of birth and adult life mortality. (Paper III & IV)

To investigate the relation between month of birth and all-cause mortality in different age spans in Sweden. (Paper III)

To investigate the relation between month of birth and cause-specific mortality in the age span 50 to 80 years. (Paper IV)

METHODS

Dr. Mikey from University of Nigeria, Enugu



BIAFRA COHORT (PAPER I)

Text published in: Hult M, Tornhammar P, Ueda P, Chima C, Edstedt Bonamy A-K, et al. (2010) Hypertension, Diabetes and Overweight: Looming Legacies of the Biafran Famine. PLoS ONE 5(10): e13582.

The Biafra famine

The Nigerian civil war broke out 6 July, 1967, after the Igbo people in the south-eastern provinces had declared independence as the Republic of Biafra. The war was a culmination of ethnic, economic, and religious tensions among the various peoples of Nigeria. Disapproving of the secession, Nigerian forces rapidly pushed the Biafrans back into a small enclave. Inflow of food to this enclave was cut off. The result was extensive famine among the Igbos, regarded as one of the great nutritional disasters of modern times[73]. The war ended on 15 January, 1970.

Of the 1 to 3 million Igbos that are estimated to have lost their lives, only a small fraction (10%) died of military violence. The majority succumbed to starvation. The nutritional emergency was most critical in the Biafran enclave, in which approximately 7 million people - mostly refugees - resided. In August 1968 the first international relief operations were launched but the amount of food provided was clearly not sufficient and the great majority of the Igbos did not get access to this relief food [74].

Settings and participants

This follow-up study took place between 27 June and 31 July, 2009. All shops at the six major market places of Enugu, the former Biafran capital, were systematically covered. People at the markets were actively contacted at their work place. The selection of participants was restricted to men and non-pregnant women who knew they were born in the southeast of Nigeria between 1965 and 1973. All subjects reported year of birth and 73% reported at least month and year. To confirm the birth year specified by the subject, we asked each person for a short review of his or her family history during the Nigerian civil war. More than 90% of eligible subjects accepted participation in the study. It was not possible to do a systematic categorization of those who declined.

Data collection was conducted by the three lead investigators (MH, PT, PU) as well as doctors and medical students from the University of Nigeria Teaching Hospital. Recruitment was performed by all team members, whereas measurements of blood pressure and p-glucose were obtained by seven specially trained investigators. Subjects were asked about level of education, current smoking, previously diagnosed hypertension or diabetes mellitus, and treatment for these conditions. Level of education was categorized as none, primary, secondary or higher education. There was no available information on birth weight or infant nutrition.

All measurements were performed according to predefined standard operating procedures. Participants were instructed to rest seated for at least five minutes before blood pressure (BP) and heart rate were measured. Two readings were taken three minutes apart in the left arm using a validated [75] automated oscillometric device (Omron M6 HEM-7001-E, Omron Corporation, Kyoto, Japan). A third reading was taken if the first and second BP differed more than five mmHg and mean systolic and diastolic values were calculated and defined as the subject's BP. Subjects having a systolic BP (SBP)

≥140 mm Hg, or a diastolic BP (DBP) ≥90 mm Hg or who had a normal blood pressure but were pharmacologically treated for hypertension (41 of 1,339 subjects), were categorized as hypertensive. Subjects having a SBP ≥160 mm Hg, and a DBP≥100 mmHg, were categorized as severely hypertensive. Random plasma glucose (p-glucose) was measured with a minimally invasive sampling technique (Abbott Freestyle Lite, Abbott Diabetes Care Ltd, Oxon, UK). A random p-glucose of 11.1 mmol/l or higher was classified as diabetes mellitus and impaired glucose tolerance (IGT) was defined as random p-glucose between 7.8 and 11.0 mmol/l . A subgroup (n = 75) reported no food intake for at least 8 hours prior to investigation. In this group, IGT was defined as p-glucose between 6.1 and 6.9 mmol/l and diabetes mellitus as a p-glucose ≥7.0 mmol/l. Height and weight were obtained from all subjects using height sticks and digital scales, respectively, and body mass index (BMI) was calculated. Overweight was defined as a BMI over 25 kg/m2 and obesity as BMI over 30 kg/m2. Waist circumference was measured with a measuring tape placed around the abdomen at the level of the upper hip bone, and considered as an indicator of central obesity.

All participants were briefly counselled about their current health status. Hypertensive or glucose intolerant subjects were referred to the University of Nigeria Teaching Hospital for further investigation and treatment.

Coal market, Enugu

Field work in Enugu. Pictures are taken with consent from study participants

Categorization according to exposure to famine

Subjects born between 1965 and 1967 were categorized as being exposed to famine in early childhood. Subjects born between 1968 and January 1970 (end of war) were categorized as being exposed to famine in fetal life and/or in infancy. Subjects born immediately after the surrender of Biafra were left uncategorized because of uncertainties in their exposure to famine and local variations in nutritional relief. Accordingly, subjects born between 1971 and 1973 and after the transitional period were categorized as being unexposed to famine.

Efforts to limit any potential bias included investigator-driven recruitment of participants (in contrast to subjects actively seeking participation in the study), direct assessments of outcomes with non-operator dependent methods and predefined standardized operational procedures applied by all data collectors.

Statistical methods

We aimed to include 630 individuals in each group, based on an assumption of a standard deviation of 19 mm Hg and a group difference in systolic blood pressure of 3 mm Hg or more, given 80% power and a statistical significance level of 0.05. Because of exhausted recruitment at the six study markets, data collection was ended when 1,339 subjects had been included in the study. All subjects were included in logistic regression analyses according to

year of birth. In group wise logistic regression analyses, the 173 subjects born during the transition from famine to nutritional relief, i.e., from February to December 1970, were excluded.

Data are presented as means (SD), proportions (%) and odds ratios (95% CI). Group differences were tested using ANOVA and chi-squared test. Logistic and linear regression analyses were used to calculate odds ratios (OR) and for evaluation of relations between exposure (fetal-infant famine), potential confounders (sex, smoking, educational level) and outcomes (blood pressure, p-glucose, and BMI).

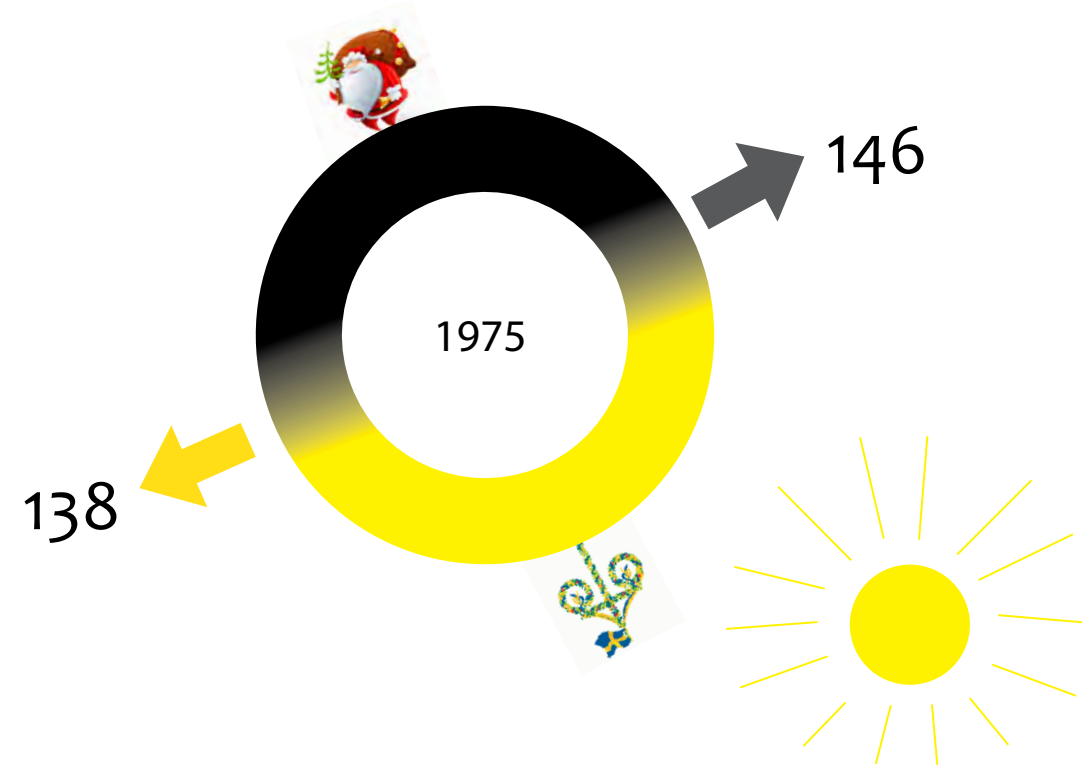
Pictures are taken with consent from study participants

NEONATAL VITAMIN D AND ADULT CARDIOVASCULAR RISK (PAPER II)

Study cohort

The Swedish phenylketonuria (PKU) Register comprises dried blood samples from screening of rare metabolic disorders in all infants born in Sweden from 1975 and onwards—a collection of approximately four million individual samples.

Starting from birth dates in February (winter) and September (summer), subjects born at hospitals in the Stockholm region (latitude 59° north) in 1975 were identified from the PKU Register. Subjects were invited to participate in the study in the order they appeared in the registry's data storage system. Birth dates ranged from 9 February – 7 March in the winter group and 14 September – 29 September in the summer group. In total, 1,305 individuals were considered for participation in the study to reach the targeted sample size of 284(see statistical methods). Reasons for non-participation included missing data in the PKU Register or no publicly available contact information (n=202), death (n=1), emigration (n=5) and failed or rejected invitation (n= 813). Response rates were not influenced by sex or month of birth; of those who did not participate, 513(50.2%) were women and 498 (48.8%) were born in the winter.



Clinical examination

Study participants were clinically examined at the Karolinska University Hospital in Stockholm between July 5 and August 30, 2010. Information about subjects' birth date, preterm birth (birth before 37th week of gestation) and postnatal age at blood sampling for metabolic screening was retrieved from the PKU Register. At enrollment in the study, participants were asked about level of education (categorized as primary, secondary or higher education), smoking, previously diagnosed diabetes mellitus or hypertension, current medications, hours of physical activity per week, weekly fish consumption and family history of diabetes mellitus.

On the day of examination, participants were asked to refrain from smoking, consumption of Swedish snuff (moist powder tobacco), intake of coffee, vitamin C or cyclooxygenase inhibitors. All measurements were performed according to predefined standard operating procedures. Aortic pulse wave velocity (PWV) was measured using AtCore Medical (www.atcormedical.com) SphygmoCor Pulse Wave

Velocity System and SphygmoCor Software according to specified procedures. Proximal site for the aortic PVW measurement was the carotid artery and distal site was the femoral artery. Participants were rested, sitting for five minutes before blood pressure was measured. Two readings were taken three minutes apart in the left and right arm respectively using a validated automated oscillometric device (Omron M6 HEM-7001-E, Omron Corporation, Kyoto, Japan). If the diastolic or systolic measurements differed more than 5 mm Hg between the measurements, the subject was rested for five minutes and a third reading in the left arm was taken. The lowest diastolic and systolic values were defined as the subject's blood pressure. Participants with a systolic blood pressure (SBP) ≥140 mm Hg or a diastolic blood pressure (DBP) ≥90 mm Hg, or who were pharmacologically treated for hypertension were categorized as hypertensive. Prehypertension was defined as 140 mm Hg >SBP≥120 mm Hg or 90 mm Hg >(DBP)≥80 mm Hg and no pharmacological treatment for hypertension.

Height and weight were measured using a measuring tape and digital scales. Body mass index (BMI) was calculated according to the formula weight (in kg) divided by height (in square meters). Overweight was defined as a BMI ≥ 25kg/m2 and obesity as BMI ≥30 kg/m2. Fasting blood glucose, blood lipids, CRP, plasma, 25(OH)D and insulin levels were measured with accredited and standardized methods at the hospital. A fasting plasma glucose level from 5.6 mmol/L to 6.9 mmol/L was defined as impaired fasting glucose according to the American Diabetes Association criteria. Subjects with a fasting plasma glucose ≥7 mmol/L or who were pharmacologically treated for diabetes mellitus were categorized as diabetic.

Measurement of neonatal vitamin D-levels

Two 3.2 mm punches were obtained from the dried neonatal blood spots of 275 out of the 284 participants. Nine of the samples could not be found in the PKU Register. 25(OH)D can exist in two forms: 25(OH)D3 and 25(OH)D2; the latter can only be obtained from a number of dietary sources and supplements. Both 25(OH)D3 and 25(OH)D2 were measured in the dried blood spot using a highly sensitive liquid chromatography tandem mass spectroscopy assay.[76] 25(OH)D2 was only detected in 29 samples (11%), at levels that were only just above the lowest level of assay quantification, mean (SD) 7.57(4.35) nmol/L. Due to uncertainties in the detecting thresholds for 25(OH)D2, only 25(OH)D3 levels were examined for the purpose of this study.

Using archived samples from Australia and Denmark, the assay has been shown to reliably detect seasonal (within year) differences in 25(OH)D3-levels, and the measurements have been demonstrated to strongly correlate with neonatal cord blood levels (r=0.86).[77] The method has been used on dried blood spots stored for more than 20 years[78] and assessed with respect to punch position, spot volume and paper type[79]. In this study, the punches were taken from the outer part of the dried blood spots.

25(OH)D3 and 25(OH)D2 are highly protein-bound steroids that are completely

The PKU Register - not the most stimulating work environment...

Focus is key!

excluded from erythrocytes[79]. Therefore, to make results comparable with existing studies, 25(OH)D3 and 25(OH)D2 concentrations were reported as adjusted sera concentrations. This requires a correction based on a standard neonatal capillary hematocrit of 0.61.[80]

The dried blood spots in this study– stored for 37 years between collection and analysis - represent the oldest clinical dried blood spot samples examined for 25(OH)D3 to date. A pilot study was therefore conducted on a random small sample of de-identified spots collected 30 years apart to assess the feasibility of this study. (6 samples from March 1980, 5 samples from September 1980, 10 samples from March 2010 and 10 samples from September 2011) Although a clear seasonality in the 25(OH)D3 levels was preserved in both archived and contemporary spots (although significant only for contemporary samples), a substantial reduction in 25(OH)D3 was seen in the archived spots (mean = 28.1, SD=11.4 v 58.6, 26.2 nmol/l , P<0.001) presumably indicating that sample degradation had occurred. This is a major reason for why samples were stratified as quintiles rather than absolute nmol/l cut-offs as described below. The investigators were blinded to the month of birth of the participants during gathering of dried blood, vitamin D measurement and clinical examination.

Statistical analyses

Blood pressure was the primary outcome assessed in this study. In order to detect a difference in systolic blood pressure of 4 mm Hg or more (SD=12 mm Hg, 80% power, significance level 0.05) between the two groups (season of birth), 142 individuals in each group were required. As secondary outcome variables may be associated with the primary outcome and with each other, no adjustment for multiple comparisons were made.

Mmm, my precious... Punches of dried blood for vitamin D measurement

Seasonal group differences in cardiovascular outcomes were compared using the t-test for continuous variables, and Fischer exact or chi square tests for categorical variables. The study cohort was categorized into quintiles of neonatal 25(OH)D3. In multiple regression analyses, the relation between neonatal 25(OH) D3 quintile and adult cardiovascular risk factors was assessed. The models were initially adjusted for sex and postnatal age at neonatal blood sampling (model 1) and then included data on maternal age at delivery, preterm birth, participants' education, exercise, fish consumption, smoking, 25(OH)D at follow-up, and family history of diabetes for outcomes related to plasma glucose (model 2). For each of the outcomes, a regression analysis was performed including the variables sex, neonatal 25(OH)D3 quintile and an interaction variable (sex*25(OH)D3 quintile). For cardiovascular risk outcomes showing significant interaction effects between sex and neonatal 25(OH)D3 quintile, sex specific analyses were conducted.

The relations between neonatal 25(OH)D3 quintile and risk of prehypertension/hypertension, overweight, obesity and impaired fasting glucose/diabetes mellitus were assessed by calculating odds ratios with 95% confidence intervals in logistic regression analyses (adjusted for confounders according to model 1 and model 2 as above). Analyses were performed using STATA/IC 11.0 (Stata Corp LP, TX, US). All tests were two-sided and p<0.05 was regarded statistically significant.

MONTH OF BIRTH AND MORTALITY (PAPER III&IV)

Parts of the text published in: Ueda P, Edstedt Bonamy A-K, Granath F, Cnattingius S (2013) Month of Birth and Mortality in Sweden: A Nation-Wide Population-Based Cohort Study. PLoS ONE 8(2): e56425. doi:10.1371/journal.pone.0056425

Study population and data sources

Data used in the studies were obtained from population-based health and administrative registries in Sweden. Cross-linkage across these registries was possible using the person-unique Personal Identification Number (PIN), assigned to all Swedish residents [81]. Using the Swedish Total Population Register, we identified all Swedish-born subjects who were living in the country on the 1st of January 1991. We included all subjects who were more than 30 years old before the end of follow up (December 31st, 2010; n=6,583,693). From the Swedish Total Population Register, we also retrieved information about dates of emigration and death. Data on subjects' educational level - categorized into primary, secondary and higher education (studies at university) - was obtained from The Education Register for the years 1990, 1995, 2000, 2005 and 2010. The highest educational level reached during the study time was defined as the subject's education.

From the Cause of Death Register, information about the underlying cause of death was obtained. Causes of death were coded according to the International Classification of Diseases (ICD). The ninth version (ICD-9) was used between 1991 and 1996, and the tenth version (ICD-10) has been used thereafter. Translation of codes between ICD 9 and ICD 10 was made using a conversion table from the Swedish

National Board of Health and Welfare [82]. Causes of death were grouped into cardiovascular diseases, infections, tumors and external causes. Subjects were followed until emigration, death, or end of follow-up (December 31st, 2010).

Statistical methods: Paper III&IV

The population was divided by month of birth into twelve groups. To adjust for cohort effects, all analyses were stratified on 10-year birth cohorts.

We assessed the association between month of birth and mortality by fitting Cox proportional hazards regression models using attained age as the underlying time scale. The analyses only included ages above 30 years. Left truncation was adjusted for by calculating an individual's age on the 1st of January 1991. Subjects contributed with person-time only in the age-spans that they belonged to during the study time.

Six percent (n=388,948) of the subjects lacked education data. We therefore compared results from crude analyses of month of birth and mortality, when including and excluding subjects with missing information on education. As the influence of month of birth on mortality risks did not essentially differ between the models in any of the studies, subjects without education data were excluded in the analyses presented in the studies. As previous studies have shown lowest mortality for subjects born in November,[36] this month was used as reference in the Cox regression analyses. Relative risks are presented as the excess hazard ratio (EHR) in per mille ((Hazard ratio – 1)*1000). Statistical analyses were conducted in SAS version 9.0 (SAS Institute, Cary, North Carolina, USA).

Statistical methods: Paper III

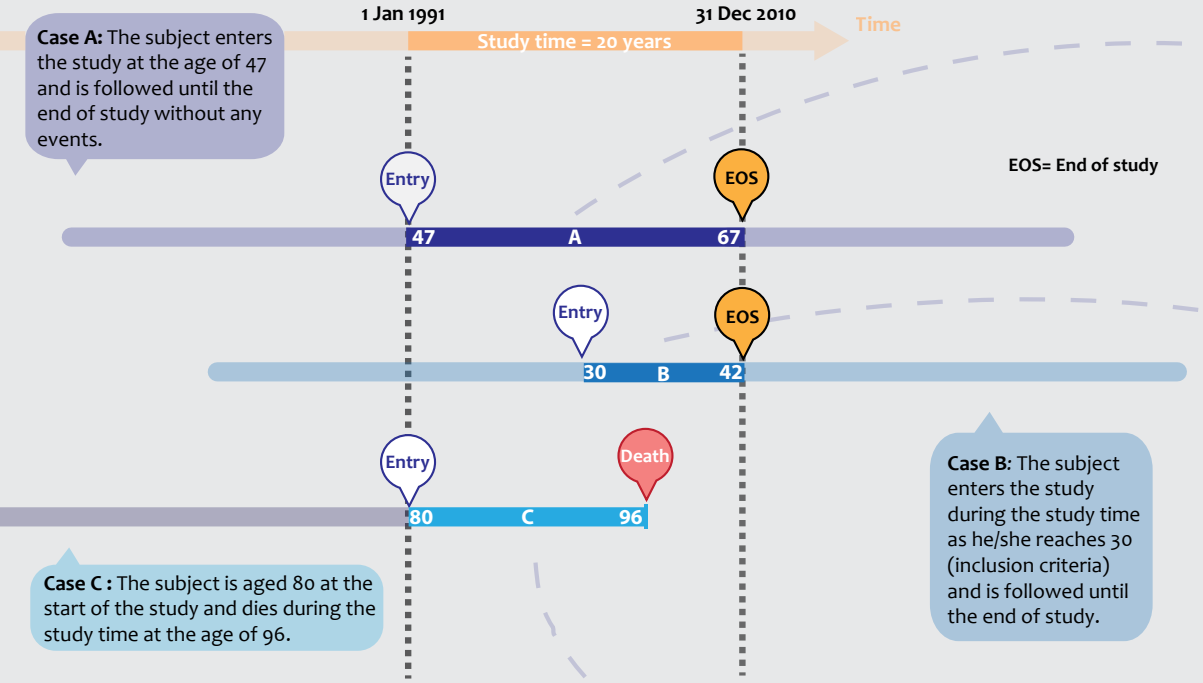
Analyses were conducted for the age-spans >30 to 50, >50 to 80 and >80 years. The crude models were followed by models adjusted for sex and education. Due to the computational burden, it was not possible to statistically test the differences in month of birth effects on mortality between the chosen age-spans.

Statistical methods (Paper IV)

Based on the results from paper III, where the association between month of birth and all-cause mortality was most pronounced in ages more than 50 to 80 years, we chose to study cause-specific mortality in this age-span. For each of the four groups of causes of death (cardiovascular diseases, infections, tumors and external causes), a crude analysis was performed, followed by a model adjusted for education and sex.

SURVIVAL ANALYSIS WITH ATTAINED AGE AS TIME SCALE

Three cases

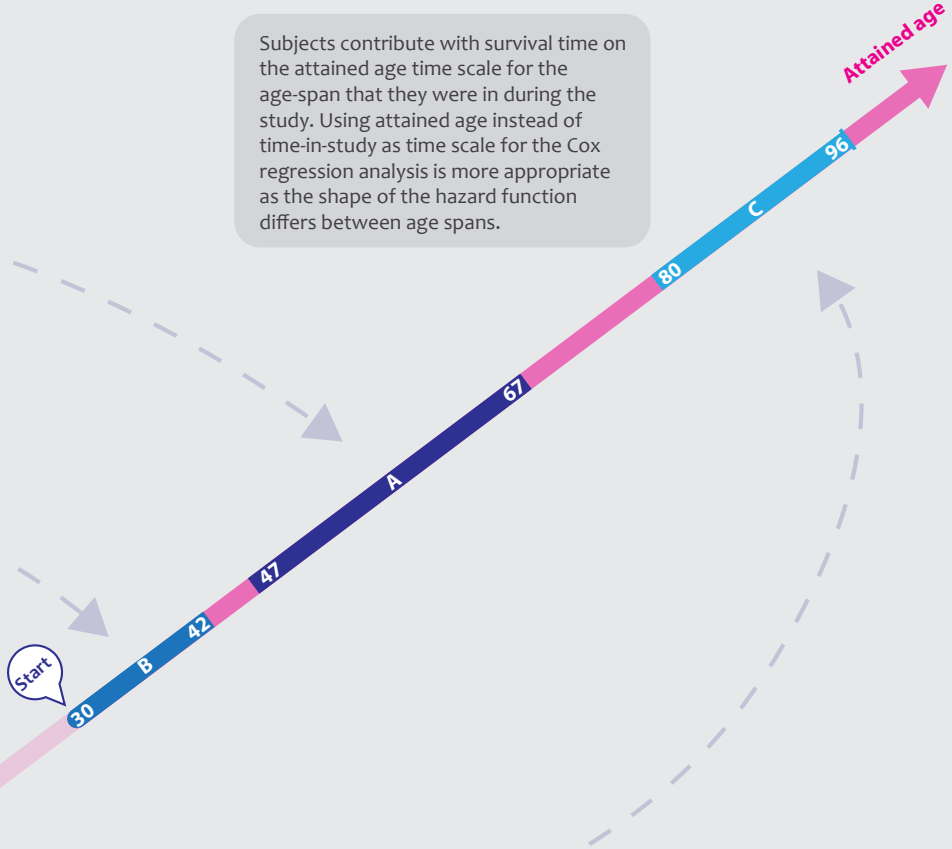


Case A: The subject enters the study at the age of 47 and is followed until the end of study without any events.

Case B: The subject enters the study during the study time as he/she reaches 30 (inclusion criteria) and is followed until the end of study.

Case C : The subject is aged 80 at the start of the study and dies during the study time at the age of 96.

Subjects contribute with survival time on the attained age time scale for the age-span that they were in during the study. Using attained age instead of time-in-study as time scale for the Cox regression analysis is more appropriate as the shape of the hazard function differs between age spans.



RESULTS

Table A2. Odds ratios (OR) and 95% confidence interval (CI) for hypertension, impaired glucose tolerance, diabetes, overweight (BMI>25 kg/m2) and obesity (BMI>30kg/m2) in 40-year-old men and women exposed to famine in early childhood, fetal-infant life, or unexposed. SBP = systolic blood pressure, DBP = diastolic blood pressure, HT=hypertension, IGT = impaired glucose tolerance. *SBP ≥160 and DBP ≥100 mmHg.

			ALL SUBJECTS N=1,166				FEMALE N=378				MALE N=788			
	N	n (%)	Crude		Adjusted for BMI		Crude		Adjusted for BMI		Crude		Adjusted for BMI	
			OR	C.I.	OR	C.I.	OR	C.I.	OR	C.I.	OR	C.I.	OR	C.I.
SBP ≥ 140	1,166	177 (15)												
Childhood	388	61 (16)	1.78	(1.19-2.68)	1.77	(1.17-2.68)	1.45	(0.65-3.28)	1.46	(0.65-3.30)	2.04	(1.26-3.31)	2.11	(1.29-3.45)
Fetal-infant	292	70 (24)	3.02	(2.01-4.52)	2.87	(1.9-4.34)	3.52	(1.63-7.60)	3.47	(1.6-7.51)	2.85	(1.75-4.64)	2.72	(1.65-4.49)
Unexposed	486	46 (9.5)	1	ref	1	ref	1	ref	1	ref	1	ref	1	ref
DBP ≥ 90	1,166	204 (18)												
Childhood	388	64(16)	1.35	(0.93-1.97)	1.30	(0.88-1.91)	1.28	(0.65-2.51)	1.20	(0.61-2.38)	1.40	(0.87-2.23)	1.42	(0.88-2.29)
Fetal-infant	292	78 (27)	2.49	(1.72-3.62)	2.28	(1.56-3.34)	2.86	(1.48-5.52)	2.72	(1.40-5.27)	2.25	(1.41-3.58)	2.06	(1.27-3.33)
Unexposed	486	62 (13)	1	ref	1	ref	1	ref	1	ref	1	ref	1	ref
Severe HT*	1,166	47 (4.0)												
Childhood	388	14 (3.6)	1.36	(0.63-2.93)	1.42	(0.65-3.13)	1.21	(0.32-4.61)	1.20	(0.31-4.59)	1.28	(0.49-3.38)	1.48	(0.54-4.03)
Fetal-infant	292	20 (6.8)	2.68	(1.31-5.46)	2.50	(1.19-5.26)	2.30	(0.65-8.10)	2.20	(0.62-7.79)	2.82	(1.18-6.73)	2.66	(1.05-6.73)
Unexposed	486	13 (2.7)	1	ref	1	ref	1	ref	1	ref	1	ref	1	ref
IGT	1,114	108 (9.7)												
Childhood	374	34 (9.1)	1.15	(0.70-1.86)	1.13	(0.69-1.83)	0.82	(0.35-1.95)	0.79	(0.33-1.90)	1.33	(0.73-2.44)	1.32	(0.72-2.42)
Fetal-infant	278	37 (13)	1.76	(1.09-2.85)	1.65	(1.02-2.69)	1.23	(0.52-2.89)	1.12	(0.47-2.66)	2.02	(1.11-3.67)	1.93	(1.05-3.52)
Unexposed	461	37 (8.0)	1	ref	1	ref	1	ref	1	ref	1	ref	1	ref
Diabetes	1,114	25 (2.2)												
Childhood	374	9 (2.4)	1.88	(0.66-5.33)	1.81	(0.64-5.15)	1.55	(0.36-6.64)	1.49	(0.35-6.44)	1.93	(0.43-8.68)	1.92	(0.43-8.69)
Fetal-infant	279	11(3.9)	3.11	(1.14-8.51)	2.56	(0.92-7.17)	2.06	(0.48-8.86)	1.85	(0.43-8.03)	3.84	(0.95-15.5)	3.15	(0.74-13.4)
Unexposed	461	6 (1.3)	1	ref	1	ref	1	ref	1	ref	1	ref	1	ref
Overweight	1,150	732 (64)												
Childhood	384	238 (62)	1.02	(0.77-1.34)			1.32	(0.72-2.42)			0.82	(0.59-1.14)		
Fetal-infant	287	199 (69)	1.41	(1.03-1.93)			2.13	(1.04-4.35)			1.19	(0.83-1.71)		
Unexposed	479	295 (62)	1	ref			1	ref			1	ref		
Obesity	1,150	253 (22)												
Childhood	384	88 (23)	1.20	(0.87-1.67)			0.95	(0.57-1.58)			1.11	(0.69-1.80)		
Fetal-infant	287	70 (24)	1.30	(0.92-1.85)			1.23	(0.72-2.12)			1.03	(0.60-1.75)		
Unexposed	479	95 (20)	1	ref			1	ref			1	ref		

BIAFRA COHORT (PAPER I)

Text published in: Hult M, Tornhammar P, Ueda P, Chima C, Edstedt Bonamy A-K, et al. (2010) Hypertension, Diabetes and Overweight: Looming Legacies of the Biafran Famine. PLoS ONE 5(10): e13582.

Age differed among the three groups due to inclusion criteria. The proportion of men varied between 66 and 74% in the three groups and was highest in the group born after the famine. Smoking occurred predominantly in men and the proportion of smokers differed in the three groups (Table A1). Educational levels were found to be distributed equally between groups (p = 0.38) and overall, no education was found in 1%, primary in 34%, secondary in 52% and higher education in 13%

Famine and risk of hypertension

SBP and DBP were higher after fetal-infant exposure to famine, as compared to the other two groups, i.e., those exposed in early childhood as well as those that were born after the famine, i.e., were unexposed (Table A1). In addition, the OR's for a SBP and DBP in the hypertensive range were significantly higher for the group exposed to fetal-infant famine (Table A2).

In linear regression analyses, SBP and DBP were associated with BMI (b= 0.49, r = 0.13, p< 0.001 and b= 0.54, r = 0.21, p< 0.001, respectively) and male sex (b = 3.3, r = 0.08, p= 0.003 and b=21.46, r = 0.06, p= 0.05, respectively), but not with smoking.

After adjusting for BMI, odds ratios for SBP and DBP in the hypertensive range were of the same magnitude as before adjustment and still significantly higher for the group exposed to fetal-infant famine. In addition, analyses stratified by sex showed that exposure to famine in fetal life and/or infancy was a risk factor for

Table A1. Subject characteristics

	Born 1965-1967 Exposed to famine in early childhood	Born 1968-January 1970 Fetal-infant famine	Born 1971-1973 Unexposed	p	*Feb-Dec 1970 Transitional period*
Number of subjects (1,339)	388	292	486		173
Sex, male	246 (66%)	189 (66%)	353 (74%)	0.01	112 (65%)
Age	43.0 (0.8)	40.5 (0.6)	37.0 (0.8)	N/A	39
Smoking, yes (%)	46 (12%)	27 (9%)	81 (17%)	0.007	19 (11%)
Education, N (%)					
none	6 (2 %)	4 (1%)	5 (1%)		4 (2%)
primary	138 (38%)	80(30%)	152 (33%)		46 (28%)
secondary	172 (48%)	152 (57%)	239 (52%)		97 (58%)
higher	44 (12%)	33 (12%)	60 (13%)		19(11%)
Current data					
Systolic blood pressure, mm Hg	125 (17)	129 (19)	122(16)	<0.001	124 (19)
Diastolic blood pressure, mm Hg	81 (11)	84(12)	79(11)	<0.001	81 (12)
Heart rate, bpm	75 (11)	76 (11)	75 (11)	0.27	77(10)
Random p-glucose, mmol/l	6.1 (1.6)	6.4 (2.0)	6.1 (1.8)	0.04	6.3 (2.6)
Weight, kg	76.2(12.9)	78.5(13.4)	77.0 (13.4)	0.08	76.3 (15.4)
Height, cm	169 (8)	169(8)	170(8)	0.034	169(8)
Waist circumference, cm	93 (11)	94(13)	91(11)	0.0011	92(12)
BMI, kg/m^2	26.7(4.7)	27.5(4.6)	26.5 (4.4)	0.016	26.6 (5.1)

Data are mean (SD) or number (%) of subjects. p-values for ANOVA or chi-squared test across groups. Data from transitional period (February 1970- December 1970) not included in the analyses. BMI=body mass index.

SBP and DBP in the hypertensive range in both women and men. The OR (95% CI) for severe hypertension in the group exposed to fetal-infant famine was 2.82 (1.18–6.73) in men and 2.30 (0.65–8.10) in women (Table A2).

Odds ratios for high SBP (>=140 mm Hg) according to each year of birth and with 1972 as reference year (OR = 1), are presented in Figure A1. The figure shows that famine in fetal-infant life (birth year 1968 and 1969) resulted in a three to four fold increase in risk of an adult SBP in the hypertensive range, both in women and men. Formal statistical testing for an interaction between early exposure to famine by sex and adult overweight was not significant, suggesting that the effects of early famine and current overweight on SBP were additive to each other.

Famine and risk of impaired glucose tolerance/diabetes

Random p-glucose was higher in adults exposed to famine in fetal-infant life (Table A1). The crude odds ratio for both IGT and diabetes was significantly higher for the group exposed to fetal-infant famine in comparison to the subjects born after the famine. Exposure to famine in early childhood did not increase the risk of IGT and diabetes in later life (Table A2).

In linear regression analyses, random p-glucose was associated with BMI (b=0.04, r=0.12, p=0.001) and male sex (b=20.41, r=0.10, p=0.001). In analyses adjusted for BMI, fetal-infant exposure to famine remained as a risk factor for IGT and diabetes in Nigerian subjects in their forties.

Stratified analyses by sex showed that the OR (95% CI) for diabetes after exposure to fetal-infant famine was 3.84 (0.95–15.5) in men and 2.06 (0.48–8.86) in women (Table A2).

Famine and risk of overweight

The group born after the famine was significantly taller than the other two groups, whereas there were no group differences in weight (Table A1). Waist circumference and BMI were higher in adults exposed to famine in fetal-infant life (Table A1). The crude odds ratio for overweight (BMI>25) was significantly higher for the group exposed to fetal-infant famine in comparison to the subjects born after the famine. Stratified analyses by sex showed that this effect was confined to women (Table A2).

NEONATAL VITAMIN D AND ADULT CARDIOVASCULAR RISK (PAPER II)

We investigated 138 subjects born in September and 146 subjects born in February/March 1975. Maternal age at delivery was significantly higher for the group born in September. Other cohort characteristics were equally distributed within the groups (Table B1).

Outcomes by month of birth

Neonatal 25(OH)D3 levels were significantly lower in the group born in February/March (31.5 vs 48.5 mmol/l, p<0.001)) whereas 25(OH)D levels at adult follow-up did not differ between the two groups

Figure A1. Odds ratios for high systolic (≥140 mmHg) blood pressure in men and women at follow-up in 2009 according to year of birth and with 1972 as reference

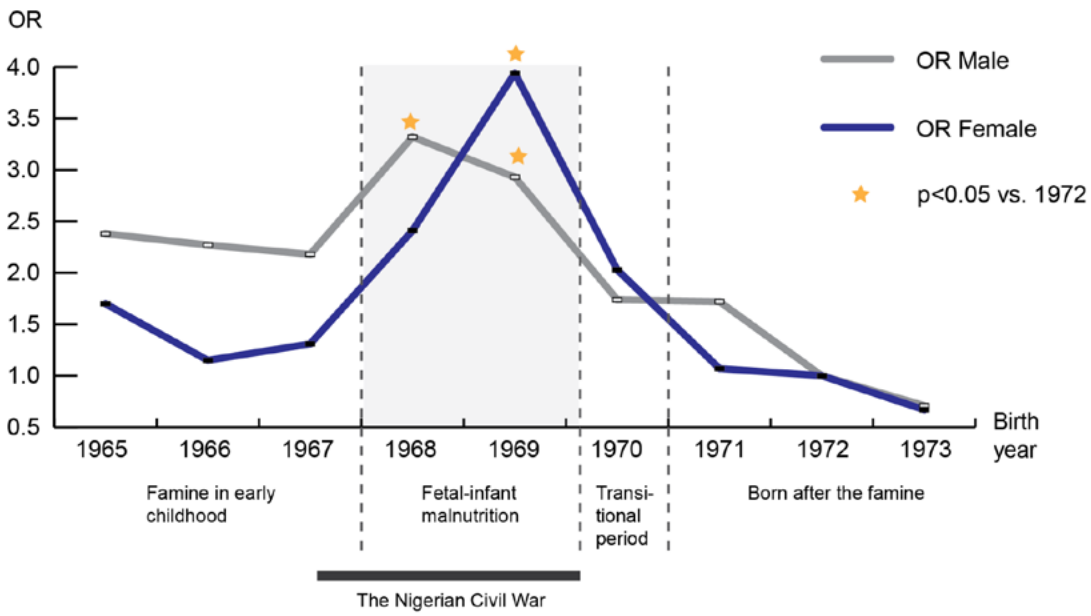


Table B1. Cohort characteristics by season of birth

	Born in Feb/Mar 1975 (n=138)		Born in Sep 1975 (n=146)		
Data at birth (1975)	*N*	Value	*N*	Value	p-value
Maternal age, years (SD)	136	28.6 (4.9)	146	27.1 (4.0)	0.005
Female sex, n (%)	138	60 (43%)	146	64 (44%)	0.95
Preterm birth, n (%)	136	4 (3%)	140	3 (2%)	0.72
Data at follow-up (2010)					
Smokers, n (%)	138	17 (12)	142	26 (18)	0.16
Fish consumption, times/week (SD)	135	1.6 (1.3)	140	1.4 (1.0)	0.44
Exercise, hours per week (SD)	135	2.2 (2.3)	140	1.9 (1.95)	0.23
Education N	138		142		0.82
Primary, n (%)		5 (3.6%)		4 (2.8%)	
Secondary, n (%)		56 (40.6%)		54 (38.0%)	
Higher, n (%)		77 (55.8%)		84 (59.2%)	
Family history of diabetes, n (%)	137	15 (10.9%)	141	15 (10.6%)	0.93

Table B2. Neonatal 25-hydroxyvitamin D_3 (25[OH]D3) quintiles and odds ratios (ORs) with 95% confidence intervals (CI) for prehypertension/hypertension, overweight, obesity and impaired fasting glucose/diabetes mellitus at the age of 35 years.

		Neonatal 25(OH)D3 quintile					
	N	1	2	3	4	5	P-value
Prehypertension/ Hypertension, n (%)		12 (21.8)	24 (42.9)	11 (20.4)	10 (18.2)	22 (40.0)	
Model 1[a], OR (95% CI)	272	1.00 (ref)	2.46 (1.00-6.00)	1.00 (0.38-2.65)	0.77 (0.29-2.05)	2.00 (0.82-4.89)	0.79
Model 2[b] OR (95% CI)	240	1.00 (ref)	2.28 (0.82-6.32)	0.80 (0.27-2.40)	0.60 (0.20-1.80)	1.78 (0.65-4.88)	0.88
Overweight, n (%)		22 (40.0)	25 (44.6)	25 (46.3)	20 (36.4)	38 (70.4)	
Model 1[a], OR (95% CI)	271	1.00 (ref)	1.15 (0.52-2.53)	1.39 (0.62-3.08)	0.90 (0.41-2.01)	3.22 (1.41-7.36)	0.027
Model 2[b] OR (95% CI)	239	1.00 (ref)	1.41 (0.56-3.54)	1.46 (0.57-3.72)	0.88 (0.35-2.20)	5.74 (2.14-15.37)	0.009
Women - Model 1[a], OR (95% CI)	119	1.00 (ref)	0.56 (0.14-2.26)	1.59 (0.49-5.18)	0.93 (0.27-3.18)	4.31 (1.13-16.45)	0.05
Women - Model 2[b] OR (95% CI)	109	1.00 (ref)	0.70 (0.14-3.52)	1.15 (0.28-4.78)	0.90 (0.21-3.81)	13.94 (2.30-84.4)	0.02
Men - Model 1[a], OR (95% CI)	152	1.00 (ref)	1.62 (0.58-4.53)	1.24 (0.42-3.67)	0.88 (0.31-2.53)	2.73 (0.97-7.70)	0.20
Men - Model 2[b] OR (95% CI)	130	1.00 (ref)	2.16 (0.60-7.77)	2.10 (0.53-8.23)	1.10 (0.30-3.98)	5.34 (1.50-18.94)	0.05
Obesity, n (%)		3 (5.5)	8 (14.3)	10 (18.5)	6 (10.9)	9 (16.7)	
Model 1[a], OR (95% CI)	271	1.00 (ref)	2.40 (0.58-9.90)	4.15 (1.06-16.22)	2.12 (0.50-9.03)	2.94 (0.74-11.68)	0.24
Model 2[b] OR (95% CI)	239	1.00 (ref)	1.13 (0.23-5.64)	2.48 (0.55-11.29)	1.59 (0.35-7.33)	2.79 (0.63-12.45)	0.15
Impaired fasting glucose/Diabetes mellitus, n (%)		5 (9.8)	8 (16.0)	4 (8.3)	7 (13.0)	4 (7.8)	
Model 1[a], OR (95% CI)	252	1.00 (ref)	1.33 (0.38-4.65)	0.84 (0.21-3.4)	1.32 (0.38-4.57)	0.66 (0.16-2.68)	0.61
Model 2[c] OR (95% CI)	237	1.00 (ref)	1.12 (0.30-4.18)	0.78 (0.18-3.28)	0.96 (0.25-3.59)	0.66 (0.15-2.90)	0.55

[a] Adjusted for sex and age at neonatal sample collection
[b] Adjusted for sex, age at neonatal sample collection, preterm birth, maternal age, education, smoking, fish per week, exercise per week and current 25(OH)D
[c] Adjusted for sex, age at neonatal sample collection, preterm birth, maternal age, education, smoking, fish per week, exercise per week, current 25(OH)D and diabetes heredity

(78.1 vs 74.4 mmol/l p=0.22). There were no significant differences between the two groups in cardiovascular risk outcomes.

Outcomes by neonatal vitamin D status

In fully adjusted multiple regression analyses (model 2), adults in the fifth (highest) neonatal 25(OH)D3 quintile had higher BMI (b=2.30, 95% CI=0.60 to 3.99) and weighed more compared to adults in the first quintile. There was a significant sex difference in the relation between neonatal 25(OH)D3 quintile and adult BMI – in women, BMI increased at a higher rate per increase in neonatal 25(OH)D3 quintile (b= -0.80, 95% CI = -1.54 to -0.05). In the sex specific analyses, women but not men in the fifth neonatal 25(OH)D3 quintile had significantly higher BMI (b=4.04, 95% CI =1.30 to 6.79) compared to those in first quintile. There were no significant associations between neonatal 25(OH)D3 quintile and other cardiovascular risk outcomes (pulse wave velocity, blood pressure, insulin, plasma glucose, blood lipids, CRP and 25(OH)D) at follow-up.

In the logistic regression analyses, adults in the fifth neonatal 25(OH)D3 quintile were more likely to be overweight compared to those in the first quintile (Table B2). In fully adjusted models, both men and women in the fifth quintile were at higher risk of being overweight, although the trend for the relation between neonatal 25(OH)D3 quintile and overweight was of borderline significance for men. No significant risk differences between the quintile groups was seen in fully adjusted models for prehypertension/hypertension, obesity or impaired fasting glucose/diabetes mellitus. (Table B2)

MONTH OF BIRTH AND MORTALITY (PAPER III&IV)

Parts of the text published in: Ueda P, Edstedt Bonamy A-K, Granath F, Cnattingius S (2013) Month of Birth and Mortality in Sweden: A Nation-Wide Population-Based Cohort Study. PLoS ONE 8(2): e56425. doi:10.1371/journal.pone.0056425

Paper III

A total of 6,194,745 subjects contributed with person-time to the study. During the time of follow-up, 1,287,927 (20.8%) of the subjects died. The proportion of the population that died during the study time increased with age-span, male sex and lower education. (Data not shown)

Month of birth was not a significant predictor of mortality in the age-span >30 to 50 years, but predicted mortality both at >50 to 80 and >80 years. Peak mortality was seen for birth month April (Excess hazard ratio (EHR) =42, 95% CI=30 to 55) in ages >50 to 80 years and for August (EHR=17, 95% CI=4 to 31) in ages >80 years. Overall, the mortality was highest for subjects born during the spring and summer months in both age-spans and lowest for subjects born in October-December (Figure C1).

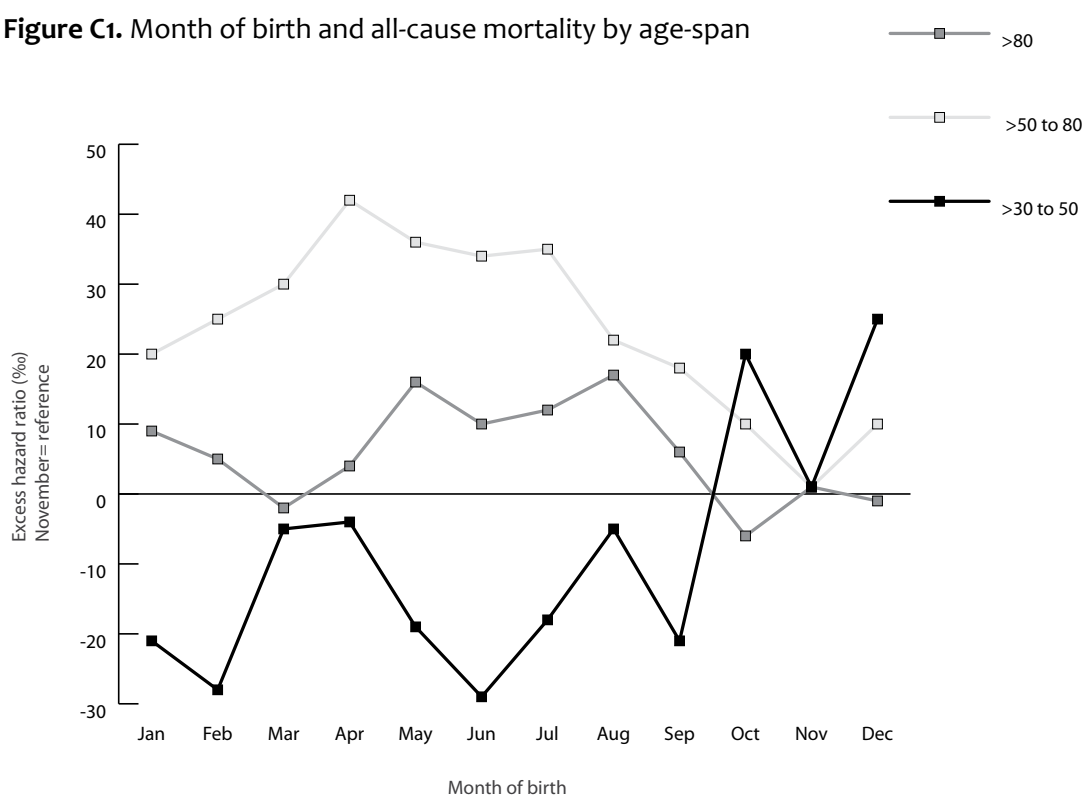
Paper IV

In total, 4,240,338 subjects were included in the study. During the 20 years of follow-up, 655,532 (15.5 %) died in the investigated age-span (more than 50 to 80 years). Cardiovascular diseases accounted for 39.5 percent of the deaths, followed by tumors (35.3%), external causes (4.6%), infections (4.2%), and other (not investigated) causes of death (16.3%). The proportion of deaths attributable to cardiovascular diseases was higher in males and in subjects

Table C1. Number of subjects, sex, education and causes of death in ages >50 to 80 years

	Total population		Cardiovascular diseases		Infections		Tumors		External causes	
	Subjects (n)	Deaths (n)	Deaths (n)	% of total deaths	Deaths (n)	% of total deaths	Deaths (n)	% of total deaths	Deaths (n)	% of total deaths
Total	4,240,338	655,532	259,106	39.5	27,747	4.2	231,684	35.3	30,091	4.6
Sex										
Male	2,080,300	389,358	167,660	43.1	15,778	4.1	124,732	32.0	20,504	5.3
Female	2,160,038	266,174	91,446	34.4	11,969	4.5	106,952	40.2	9,587	3.6
Education										
Primary	1,788,988	389,895	166,069	42.6	17,972	4.6	126,134	32.4	15,483	4.0
Secondary	1,590,157	198,185	71,777	36.2	7,624	3.8	75,672	38.2	10,481	5.3
Higher	861,193	67,452	21,260	31.5	2,151	3.2	29,878	44.3	4,127	6.1

Figure C1. Month of birth and all-cause mortality by age-span



with low education, whereas deaths from tumors were more common in females and in highly educated subjects. (Table C1)

Month of birth and cardiovascular mortality

Month of birth was a significant predictor of cardiovascular mortality in the crude model and adjusting for sex and education did not attenuate this association. Compared to subjects born in November, the risk of cardiovascular mortality was significantly increased among subjects born from January through August, with a peak in March/April (EHR=66, 95% CI=45 to 86) (Figure D1 on page 57).

Month of birth and mortality from infections

There was a significant association between month of birth and death from infections in the crude and in the adjusted analyses (p for trend=0.027). In the model adjusted for education and sex, a lower mortality risk of the birth months November to January was followed by a plateau between February and August and a spike in September (EHR=108, 95% CI=46 to 175).

Month of birth and mortality from tumors and external causes

There were no significant trends for the relation between month of birth and mortality risk due to tumors or external causes.

Photo: Mr Kang Le

DISCUSSION

BIAFRA COHORT (PAPER I)

Parts of the text published in: Hult M, Tornhammar P, Ueda P, Chima C, Edstedt Bonamy A-K, et al. (2010) Hypertension, Diabetes and Overweight: Looming Legacies of the Biafran Famine. PLoS ONE 5(10): e13582.

This study showed higher BP, higher p-glucose and higher weight in middle-aged Nigerian people exposed to severe undernutrition in utero and/or in infancy. Comparing unexposed offspring with that of starving pregnant women, fetal infant undernutrition was associated with significant increases in the prevalence of hypertension and impaired glucose tolerance or diabetes. Famine in early childhood was also associated with an increased prevalence of adult blood pressure in the hypertensive range. Given the additive effects of early famine and adult overweight, early undernutrition followed by later overnutrition seem to provide two fundaments for the adverse metabolic and cardiovascular outcomes seen in today's Nigeria, and as also previously described in an Indian cohort [83]

Comparison with previous studies

Our results are in line with previous epidemiological and experimental studies suggesting that fetal undernutrition contributes significantly to cardiovascular disease risk in adult life [84–86]. In contrast to observations in European birth cohorts [8, 11, 13, 14, 85, 87–91], the effects of fetal undernutrition seem to be more pronounced and emerge at an earlier age in this sub-Saharan cohort. These differences could reflect variations in exposure and population differences. Such an explanation may not necessarily be confined to susceptibility for and degree of fetal-infant undernutrition alone. Accelerated growth in later childhood[20, 90] and a high BMI in adult life have previously been found to have a stronger adverse effect on hypertension [92, 93] and diabetes [8, 11] in people who were small at birth. The mismatch between the environment that people in urban Nigeria now live in, characterized by a high-calorie-high-carbohydrate diet, and the one within which they evolved during the Biafra famine, may therefore be largely responsible for the present increase in disease risks [6].

Our finding of a higher current weight and waist circumference, and a higher BMI in adults exposed to fetal-infant famine (Table A1) imply that weight gain later in childhood may be in the pathway between early under-nutrition and later metabolic syndrome. Such an explanation may also be valid for the finding that there was no association between moderate to severe malnutrition in early life and glucose tolerance and blood pressure in 35-year old Gambians (predominantly women) who remained fit and lean [94]. However, as we found no attenuation of the excess odds for adult hypertension and impaired glucose tolerance after adjusting for current BMI (Table A2), the association between fetal-infant famine and adult metabolic syndrome cannot be attributed to accelerated childhood weight gain alone [95].

Gender differences in developmental programming have previously been described [20]. In our study, both men and women exposed to fetal-infant undernutrition exhibited higher odds ratios for elevated blood pressure at follow-up. An excess risk of IGT was also found in men whereas power limitations unable conclusions regarding the risk of IGT in women exposed to early famine. Conversely, a significant excess risk of overweight was only seen in women exposed to fetal-infant undernutrition. Although there are reports of increased susceptibility for fetal programming of BP in males [20, 91], sex differences in the association between birth weight and BP have been questioned [96]. Experimental data suggest possible sex specific mechanisms in fetal programming of insulin secretion and insulin resistance [97], mechanisms that may be relevant in explaining our findings of gender differences in glucose tolerance.

Strengths and weaknesses

The strengths of this study include the design with prospectively set inclusion criteria and active enrolment of a large cohort of customers and traders at markets, i.e., places where many people in the urban areas gather and work, as other sectors of employment and sites for purchasing of everyday goods are not very developed in this part of the world. Thus, we believe that the study cohort is representative for urban settings in sub-Saharan Africa and at highest risk of the present epidemic in lifestyle-related diseases. Categorization was based on date of birth – i.e., exposure to famine in early life. In addition, since subjects born in the transitional period were excluded, there was no late gestational overlap with famine in the unexposed group. The follow-up time was sufficiently long to establish relations to outcomes that are directly related to adult cardiovascular disease. Finally, we addressed the possibility that smoking confounded our results [98].

Although heritability for birth weight has been estimated to range from 25% to 40% [99], and although some experimental data suggest that birth weight may fail to reflect intrauterine factors associated with later disease risk [100], birth weight is the most commonly used proxy for fetal

Watch out for the Okadas

undernutrition. Therefore, a limitation of our study, shared with other famine studies [88, 91], is the lack of anthropometric data at birth and in infancy. There are no records from which these data can be retrieved. In addition, we have no data on mother and infant nutrition. Given that inflow of food to Biafra was cut off, it can be assumed that access to infant formula was extremely limited and that most infants were exclusively breastfed. It is likely that survival of the healthiest pregnant women occurred during the Biafran famine. The most severe cases of fetal and infant undernutrition are also likely to have died prior to follow-up, either in utero, in early childhood, or from the increasing cardiovascular morbidity reported from adults residing in the area [101].

Some misclassification may have occurred as the nutritional situation of Biafra gradually deteriorated, making it difficult to pinpoint the start of famine. Before the war, Biafra differed from most developing nations because it had a good supply of food and water, and sound public health policies with many physicians, nurses, hospitals, and clinics [102]. Already months after the war, there are reports of vast improvement in the nutritional situation [73]. Our results are therefore most likely reflecting differences in adult health outcomes after severe early famine as compared to a significantly better nutritional situation in early life.

The external validity of data from a convenience sample from market places can also be discussed. The method of recruiting participants will have missed subsistence farmers and others not attending the markets, whose nutritional status and other factors are likely to differ from market people. We note that the prevalence of hypertension, diabetes and obesity reported herein are similar to those reported from comparable urban and rural Nigerian cohorts [103, 104]. The study design, i.e., comparing long-term outcomes in people born before, during or after famine, has previously been used [8, 11, 13, 87]. By inclusion, the subjects in the unexposed group were youngest. As the prevalence of hypertension and glucose intolerance increases with age, some associations with birth year might be expected. However, given the size of the effect and that the OR's for all outcomes after fetal-infant famine were significantly lower not only in younger, but also in older people, trends in disease-risks over time and cohort effects cannot be the only explanation for our findings.

Previous studies indicate that a nutritional insult – during gestation or the first few months of postnatal life - may be important for later outcome and disease risk [11, 13]. Although the resolution and exposure data of this study do not allow for a detailed analysis of the timing of the insult, the striking dose-response effect found between birth during years of famine and over-risk of hypertension in adult life (Figure A1) indicates a causal relationship.

The Biafran famine was characterized by a severe scarcity of proteins, manifested in the vast number of infants and children suffering from kwashiorkor [74]. Experimental models suggest that protein deficit in utero may program abnormal glucose homeostasis and vascular endothelial dysfunction, whereas results are less consistent with regard to programming of high blood pressure [86, 100]. Besides the nutritional insult, pregnant women in former Biafra were living under conditions of war. Such stress for mothers and infants could also contribute to higher blood pressure in later life [105]

Relevance

On a population level, a 3.3 mm Hg increase in mean SBP and a 2 mm Hg in DBP can be translated into an estimated increase in cardiovascular deaths by 25% and stroke by 32% [106]. Given the combination of large blood pressure effects and increased rates of glucose intolerance resting on a basis of prevalent obesity before middle age, which is characteristic for Nigeria today, it is not surprising that disability and deaths from stroke and coronary heart disease are rapidly increasing.

To adequately address this trend in lifestyle-related diseases on a community level requires a developed health care infrastructure providing life-long treatment and follow-up. The increasing burden of chronic diseases therefore poses a massive challenge to the already crippled health care systems of sub-Saharan Africa.

The findings from this study underscore the potential importance of prevention of fetal and infant undernutrition to limit the increase of non-communicable diseases in many African countries.

It has to be noted however, that the severe malnutrition and stress characterizing the Biafra famine may not be akin to the type of environment that people in this region are exposed to today. Therefore, the argument that improving nutritional status among pregnant women would be of prioritized importance for preventing lifestyle-related diseases in the offspring may be questionable, if based only on the data from this study. Given the potential for more immediate improvements in pregnancy-related outcomes possibly obtained from such interventions, the findings from this study may provide yet another argument for investments in maternal health in the developing world.

NEONATAL VITAMIN D AND ADULT CARDIOVASCULAR RISK (PAPER II)

This is the first study assessing the relation between fetal-neonatal vitamin D status and cardiovascular and metabolic risk in adult age. The findings should be interpreted with consideration for the multiple outcomes assessed. Birth after winter and low neonatal 25(OH)D3 status were not associated with adverse cardiovascular risk profile such as aortic stiffening, increased blood pressure and blood lipids, impaired fasting glucose, elevated CRP or low vitamin D in adult life. Contrary to our hypothesis, we found that women with the highest 25(OH)D3 concentrations at birth had a significantly higher BMI at the age of 35. In addition, both men and women with the highest neonatal 25(OH)D3 concentrations were at an increased risk of being overweight, although the association was most pronounced in women.

Neonatal vitamin D and cardiovascular outcomes

The lack of an association between neonatal 25(OH)D3 status and cardiovascular outcomes - including aortic pulse wave velocity and blood pressure - was in broad accordance with studies in children. In an Indian study, blood pressure in children at ages 5 and 9 years was not predicted by third trimester vitamin D concentrations in the mother.[65] Also in a British study, no associations were found between maternal vitamin D status during late pregnancy and the child's blood pressure, pulse wave velocity, carotid intima-media thickness or measures of cardiac structures at 6 years of age.[107]

Neonatal vitamin D and body composition

Vitamin D is a determinant of body composition in fetal life. It has been shown that maternal vitamin D deficiency during pregnancy is associated with small for gestational age infants.[108] In an Indian study, arm muscle area in the offspring – both at 5 and 9 years-of-age – decreased with lower third trimester vitamin D concentrations in the mother.[65]

The results from this study imply that higher concentrations of neonatal 25(OH)D3 may be associated with higher BMI in women and elevated risk of being overweight in both men and women at the age of 35. There are previous reports of age and population-specific associations between early life vitamin D status and later body fat mass. Vitamin D deficiency during pregnancy in Indian mothers was associated with higher percentage of body fat and lower percentage of fat free mass in their 5-year-old sons but not in their daughters. In accordance with our findings, BMI at 5 years of age was positively associated with maternal vitamin D status during pregnancy. However, when the children had reached 9.5 years of age, these associations could no longer be seen.[65] In a study from the UK, higher maternal vitamin D in late pregnancy was associated with higher fat mass in the newborn infant. At later follow-up at 6-years-of-age, the direction of this relation had changed so that lower maternal vitamin D concentrations were associated with an increased fat mass in the offspring.[66] In contrast, another British study showed no association between maternal vitamin D during pregnancy and BMI at the age of 10.[107]

We used BMI as a proxy for body fat mass. BMI is a controversial measure for diagnosing adverse body composition as it may not reflect body fat distribution or adiposity.[110, 111] It could be speculated that mothers with high concentrations of vitamin D during pregnancy give birth to infants of larger size that are at higher risk of being overweight in adult age, but not necessarily due to increased adipose tissue or body fat percentage. Given the varying and partly conflicting findings from different populations and ages, further investigations of the link between early life vitamin D and later body size and composition are warranted.

Neonatal vitamin D and glucose metabolism, insulin and blood lipids

Indian children whose mothers had been vitamin D deficient during pregnancy exhibited higher fasting insulin levels and higher insulin resistance at 9.5 years of age.[65] In our study, fasting insulin was not associated with neonatal 25(OH)D3 quintile and insulin resistance was not measured. In the same Indian study, boys of vitamin D deficient mothers during the index pregnancy had higher levels of HDL[65]; another finding that was not replicated in our Swedish cohort of subjects aged 35 years.

Strengths and weaknesses

We had access to a homogenous cohort born and living at a latitude where sunlight exposure differs greatly between seasons. By measuring vitamin D at follow-up we could partly control for the possibility that neonatal 25(OH)D3 is an indicator for life course vitamin D exposure through shared genetic and lifestyle factors between the

photo: Nicolas Raymond. www.freestock.ca

mother and the child. A comprehensive test battery of clinically relevant outcome measures were chosen to – alone or in combination – reflect future risk of cardiovascular events.

There may be associations between neonatal 25(OH)D3 status and adult cardiovascular risk outcomes that were not detected in this study due to the limited sample size. Other limitations include lack of data on body composition at follow-up, birth weight, and maternal characteristics, including BMI and vitamin D supplementation. Albeit the long follow-up in relation to previous studies, our participants had not reached an age in which cardiovascular disease and diabetes become more prevalent. Due to potential degradation during storage time, the neonatal 25(OH) D3 concentrations could not be translated to clinically relevant cutoffs. As the study setting was limited to Stockholm, and as the study cohort constituted of subjects born in the end of either the winter or the summer, the findings may not be strictly generalizable to other birth places or birth months.

We considered 1,305 subjects for participation in the study to reach the target sample size of 284. Sex and season of birth were distributed similarly among those who did not participate in the study compared to those who did. It can be hypothesized that subjects who chose to participate were more health conscious and thus healthier compared to the general population.[112, 113] It is however difficult to see how differences in response rates would have created biased associations in this study. The rate of individuals who were overweight in our study was comparable to that reported in a population health survey [114] whereas blood pressure levels seen in this study were slightly lower than in reports from Swedish populations of similar or higher ages.[115]

MONTH OF BIRTH AND MORTALITY (PAPER III&IV)

Parts of the text published in: Ueda P, Edstedt Bonamy A-K, Granath F, Cnattingius S (2013) Month of Birth and Mortality in Sweden: A Nation-Wide Population-Based Cohort Study. PLoS ONE 8(2): e56425.

Month of birth and all-cause mortality

In paper III, we found that month of birth was a significant predictor of all-cause mortality in the age-spans >30, >50 to 80 and >80 years. The highest mortality was seen for people born in the spring/summer, peaking in April (>50 to 80 years, adjusted model) or August (>80 years), with a corresponding trough in the autumn. In the age-span >30 to 50 years, results were inconclusive.

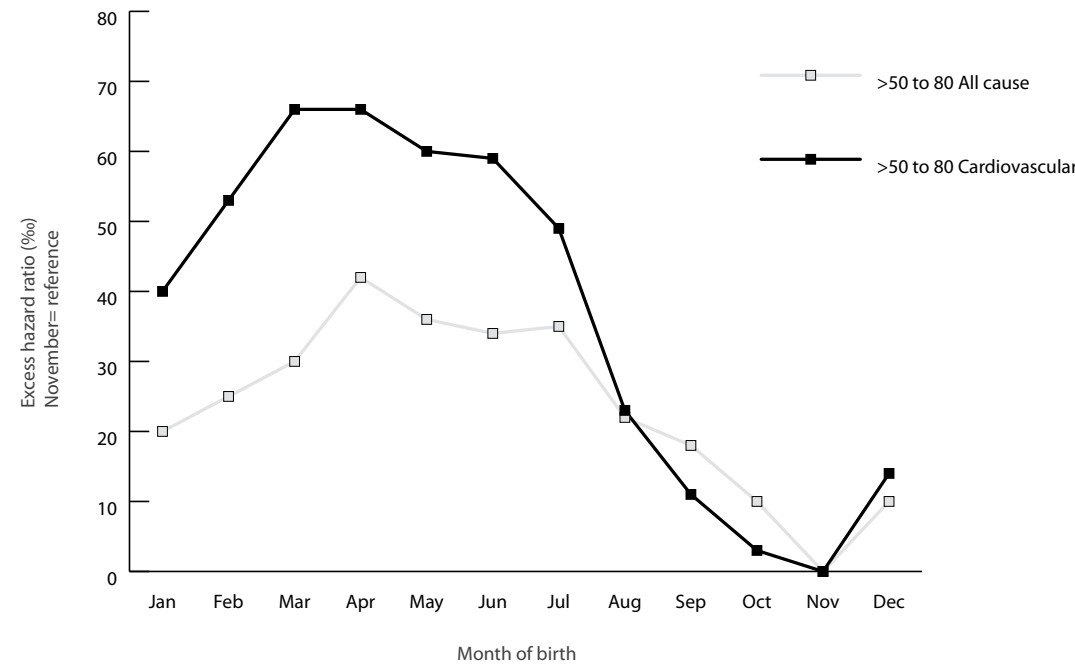
As for the ages above 50 years, the findings of this study are in broad agreement with previous reports from the northern hemisphere: autumn-born have a survival advantage compared to those born in the spring and summer. Seemingly, these effects were more pronounced in the age-span >50 to 80 years compared to >80 years. One possible explanation for this is selective mortality: irrespective of month of birth, the frailer will die first. With increasing age, the remaining population will constitute an increasingly homogenous group of robust individuals, and differences in mortality risk by month of birth will therefore gradually diminish.[35] In the youngest age-span of this study (>30 to 50 years), suicide, accidents and other causes of death related to social factors are more prominent[116]. Apart from the relatively low number of deaths occurring in this age-span compared to older ages, different pathways between month of birth and mortality could explain the inconclusive and different month of birth-pattern seen in ages >30 to 50 years as compared to >50 to 80 and >80 years.

Month of birth and cardiovascular mortality

In study IV, the association between month of birth and risk of cardiovascular mortality in ages 50 to 80 years, largely resembled the association between month of birth and all-cause mortality in the same age-span in study III. As the effects of month of birth were more pronounced for cardiovascular than for all-cause mortality, it can be assumed that the observed associations between month of birth and all-cause mortality are largely attributable to cardiovascular deaths (Figure D1).

Our results are also partly supported by results from other studies. In a cross-sectional study of more than 2,5 million Germans who died of cardiovascular diseases, subjects born from October to December reached the highest age at death[38] and the lowest age at death was seen for subjects born in May and June. Using a similar cross-sectional approach, a U.S. study including over 15 million death records, also found the lowest age at death from cardiovascular disease among subjects born in May and June.[35] In our study, we found the highest risk of cardiovascular mortality in birth months slightly earlier (March-April) in the year. Sweden is situated at higher latitudes (between the 55th and the 70th parallel), compared to Germany (47 – 55) and the U.S (24 –50). It can be speculated that certain exposures related to season prevail at different timings in Sweden compared to Germany and the U.S.

Figure D1. Cardiovascular and all-cause mortality in ages 50-80 years by month of birth



As mentioned earlier, the association between month of birth and adult lifespan has been attributed to seasonal fluctuations in nutrition and infections.[35] In the first half of the 20th century, when the population in this study was born, seasonality in nutrition availability in Sweden was more manifest than today. Compared to mothers to infants born at the end of the winter season, mothers to infants born in the fall may have had a more favorable nutritional status, especially during the last trimester when peak growth in the fetus occurs.[117] The DOHaD hypothesis suggests a link between early life nutrition and cardiovascular health and mortality in adulthood.[7, 18, 118] The focus of this thesis - the potential role of vitamin D for adult life cardiovascular risk – is discussed in more detail below.

Strengths and weaknesses

Study III, comprising observations from over 6 million subjects, is the largest longitudinal population-based study assessing the relation between month of birth and mortality in adult age. It is also the first study in the field including longitudinal data from age-spans below 50 years. Study IV is the largest longitudinal study to date assessing the relation between month of birth and cause-specific mortality in adult age. By excluding people born outside Sweden, we were able to create a homogenous study population with respect to seasonally dependent early life exposures as well as country-specific social factors.

Socioeconomic status - in this study indicated by educational level - is of importance to consider in month of birth studies. Socioeconomic status is a life course factor affecting disease and mortality risks. Preferences regarding the timing of having children may vary between couples of different socioeconomic groups. Month of birth-patterns in mortality may simply reflect such socioeconomic differences in seasonality of birth [119, 120], and socioeconomic factors are therefore potential confounders. In our study, those born in the first half of the year were more likely to have a higher education than subjects born in the second half of the year. However, the association between month of birth and mortality remained after controlling for education. Moreover, educational level of an individual is closely linked to the educational level of the parents. [121, 122] As parents in low socioeconomic groups may have been more affected by seasonal fluctuations in nutrition availability in the beginning of the 20th century than parents in high socioeconomic groups, education can also be seen as an indicator for the perinatal environment. The effects of month of birth on adult mortality would in this case interact with education. We examined interactions between month of birth and education with respect to mortality and the findings were not supportive of this hypothesis (paper III).

Generalizing the findings from these studies to other parts of the world may not be strictly possible, given the study setting being confined to Sweden, the Swedish climate and its specific seasonal exposures. However, our findings regarding the older age-spans are in line with other reports from the northern hemisphere.

Due to the large number of subjects included in this study, we were able to detect very small risk differences between subjects born in different months that are hard to translate into biological or clinical relevant measures. The relevance of this study could thus be considered as providing clues for the identification of early life exposures of potential public health interest. Severe and widespread infections as well as nutritional deficiencies were common at the time when many of the included subjects were born.[123, 124] It remains to be investigated whether the month of birth-effects on mortality risk are present for people born in Sweden today, when the burden of infections and malnutrition has substantially decreased.

Country-specific seasonal exposure of Sweden

Do the month of birth findings on mortality support the early life vitamin D hypothesis?

In paper II, there were no significant differences between subjects born in the end of the winter and the end of the summer with respect to the investigated cardiovascular risk markers at the age of 35 years. In paper IV however, cardiovascular mortality risk between 50 and <80 years was significantly higher in individuals born during the spring as compared to the autumn. This could possibly imply that low vitamin D during the last trimester is of importance to late life mortality.

However, as the reported differences in mortality risk were very small it may not be surprising that no differences in cardiovascular and metabolic risks according to month of birth were observed in the 35-year-old cohort of paper II. Study II included only participants born in the months of February/March and September. Thus, it cannot be precluded that differences in cardiovascular risk may prevail between individuals born in November and March/April; the birth months between which the largest differences in mortality were observed in paper III and IV. In hindsight, such a study design, based

on the findings from paper III and IV may have been preferred for paper II. As the study participants in paper II were only 35 years of age, it may also be a possibility that adverse cardiovascular risk associated with season of birth or early life vitamin D status, will emerge in more advanced ages.

However, as lower vitamin D concentrations at birth were not associated with increased cardiovascular risk in paper II, or in previous studies on children[65, 67], data do not support that vitamin D status at birth underlie the association between month of birth and adult age cardiovascular mortality.

Moreover, paper II indicated that high vitamin D concentrations at birth may be associated with higher adult risk of overweight. If such an association exists, and if the risk of overweight would be translated into increased health complications on population level, an elevated mortality risk among individuals born in the end of the summer when vitamin D concentrations in populations peak may have been detected in paper III and IV. Such a month of birth pattern for mortality risk was however not present.

To conclude, although there were significant differences in mortality depending on month of birth, the effect sizes were very small. Thus, taken together, the results in papers II, III and IV suggest that vitamin D concentrations at birth may not be of sizeable importance to cardiovascular health in adult age.

A beautiful hypothesis rejected?

The beautiful and simple hypotheses garner more attention than the complex and multifactorial ones. The hypothesis that low vitamin D status during gestation would increase risk of cardiovascular disease in adult age is an intriguing one.

Sweden would be the ideal setting for studying this hypothesis. If it would have held true, substantial differences in adult age cardiovascular mortality by month of birth would have been observed on population level in this country, where there are vast differences in sunlight hours between the seasons. Further, the PKU-biobank provides, in combination with the personal identification number, an excellent opportunity to prospectively investigate the relation between vitamin D status at birth and later cardiovascular outcomes.

Had this hypothesis been true, it could have been speculated that populations are predisposed to cardiovascular disease by early life vitamin D deficiency. It could partly explain the north-to-south gradient in cardiovascular disease observed in Europe [125], which previously has been attributed to lifestyle factors such as the Mediterranean diet. Furthermore, in low and middle income countries, people are spending increasing amounts of time indoors due to Westernized lifestyle and urbanization. Thus, this hypothesis would have been relevant, also in the context of the accelerating epidemic of lifestyle-related disease in these countries. However, the results from this thesis indicate that this hypothesis may not be true.

Kids nowadays.... do not spend much time in the sun. Does it matter for cardiovascular health?

Photo: Mr Kang Le

CONCLUSIONS

Fetal and infant undernutrition was associated with significantly increased risk of hypertension, impaired glucose tolerance and overweight in 40-year-old Nigerians. The findings underscore the potential importance of maternal nutrition during pregnancy for the ongoing epidemic of lifestyle-related disease in sub-Saharan Africa.

Birth in the end of the winter and low neonatal vitamin D status were not associated with adverse cardiovascular risk at the age of 35 in Sweden.

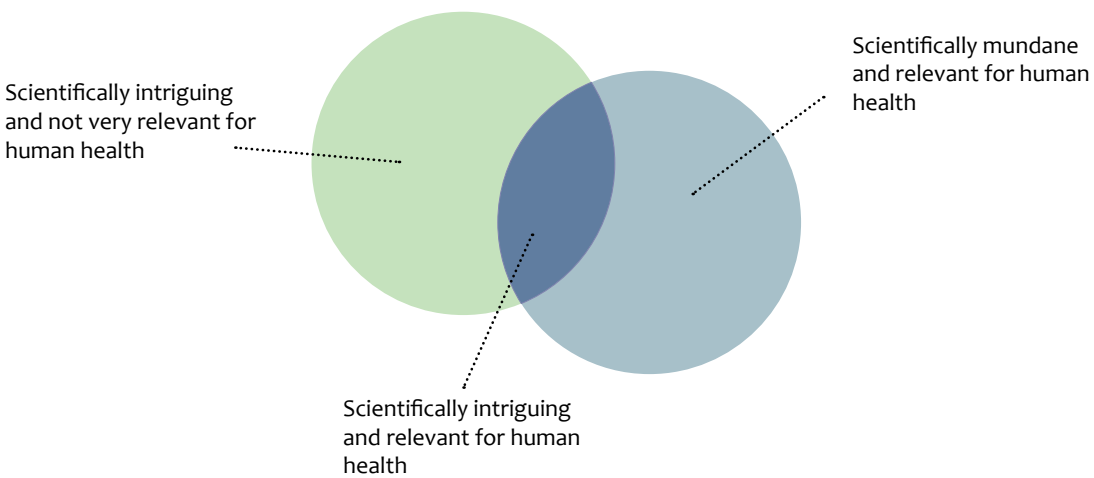
Women born with the highest neonatal vitamin D concentrations had higher BMI, and both men and women with high neonatal vitamin D concentrations were at increased risk of being overweight at the age of 35.

Spring/summer-born were shown to die sooner than autumn-born in ages 50 years and above in Sweden.

Cardiovascular mortality in ages 50 to 80 years was increased in subjects born during the spring as compared to those born during the autumn, but the risk increase was small. In combination with the findings that neonatal vitamin D status does not correlate with cardiovascular risk outcomes at age 35, the results of the present study do not support that vitamin D status at birth is of sizeable importance to adult cardiovascular health.

SUGGESTIONS ON FUTURE STUDIES

As widely agreed upon, there are two valid reasons for spending resources on medical research: scientific curiosity and real-life applications of the gained information for the improvement of human health. Ideally (for researchers), these two overlap. The suggested study topics are categorized according to the figure below.



Scientifically intriguing and potentially relevant for human health

Evaluations of the cardiovascular risk attributable to early life malnutrition in comparison to adult age cardiovascular risk factors in Sub-Saharan Africa would be relevant to assess.

Now that we have shown that neonatal vitamin D and its relation to adult age health can be assessed using the Swedish PKU-register and personal identification number, a number of hypotheses regarding early life vitamin D and later health outcomes can be examined in more detail than was possible before. Among the diseases possibly linked to early life vitamin D, we have multiple sclerosis, schizophrenia, certain brain tumors in children, wheezing, diabetes type I and autism.

The conflicting findings regarding vitamin D in early life and later body composition warrants further investigation of vitamin D levels during different periods in life and potential links to body size and composition in adult age for different populations.

Randomized controlled trials examining effects of vitamin D supplementation during pregnancy on immediate and long term health outcomes in the child. (Reportedly, this is underway)

Vitamin D production and other effects of sunlight exposure on skin are highly interesting topics put in the context of human evolution. Human skin pigmentation is adaptive and labile. Skin coloration levels have changed several times during the evolution.[126] An appropriate level of melanin pigmentation protecting against harmful effects of UV-radiation such as sunburn and skin cancer, which at the same time does not block too much of its beneficial effects, seems to be of substantial importance for survival. This importance is manifested in the variation of skin pigmentation across indigenous human populations at different latitudes.[126] Provided the rapid urbanization seen globally, the increased time spent indoors that come with economic growth and the high mobility of people across the globe, it would be both relevant and highly interesting to examine if/how low sunlight exposure affects health in different groups of people residing in different environments. Vitamin D may not be the main culprit underlying associations between sunlight exposure and health.

Evaluations of nutritional programs for optimized immediate and long term health outcomes among pregnant women and their children in low-income settings.

Scientifically mundane and potentially relevant for human health

Designing and evaluating programs to increase awareness about lifestyle-related diseases in Sub-Saharan Africa in order to prevent the epidemic on society and community level.

Looking at figure C1, there is a statistically insignificant trend towards increased mortality risk in ages 30-50 among people born late in the year. It has been observed that

children born close before the cut-off of the schooling year attain poorer educational outcomes than their relatively older peers. [127,128] The so called "birthday effect" is thought to stem from the differences in performance due to relative age being consolidated with time as better educational opportunities are presented to those who perform well within their age-group in childhood and adolescence. As the causes of death in younger ages are much associated with social factors, it would be of relevance to assess whether the "birthday effect" is present in the Swedish population (where the school year cut-off is between December and January) and if it is linked to adverse health outcomes among individuals born late in the year.

Another follow-up of the study participants in paper II. Would there be associations between neonatal vitamin D and cardiovascular risk emerging in more advanced ages?

Scientifically intriguing and not very relevant for human health

To study how and if the effects of the month of birth on adult age mortality in Sweden vary with latitude. This would give information for speculations regarding the underlying mechanisms for the differences in lifespan by month of birth. Such a study should be adjusted for socioeconomic and lifestyle-related differences between the north and south of Sweden.

It would also be interesting to investigate whether the month of birth pattern for adult mortality is present in more recent cohorts than those investigated in this thesis. If the hypothesis that seasonality in infections or availability of nutrition underlie the observed patterns in the older cohorts holds true, more recent cohorts would be less affected.

Intergenerational effects of early life malnutrition. Are the effects of early life malnutrition on cardiovascular risk passed on (through epigenetic changes?) to the next generation, and to the next?

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ACKNOWLEDGEMENTS & AFTERWORD

On Per and Martin and loneliness

In the beginning of times (i.e. 2008), this PhD-project wasn't a PhD-project. It was merely an effort launched by three medical students and friends as an excuse to explore the world. We wanted to go to Africa and we wanted a mission. So we came up with this project and the rest is... not even close to history, but at least a decent adventure in our small small lives and hopefully, a relevant contribution to epidemiology. The thesis has been designed together with Martin Hult and Per Tornhammar and the first two parts (Biafra cohort and neonatal vitamin D), we executed together.

When looking back at this PhD-project, I think about our days in House 75 at Karolinska Campus where we planned our studies constantly overdosed with caffeine, our never-ending field work in Nigeria hustling around at the markets and playing football with our Nigerian friends and the merciless summer of clinical investigations and searching for dried blood spots in a sunlight-deprived basement in Huddinge. I remember this time as the most intense and laughter-dense periods of life. These projects and the extensive data gathering under harsh conditions that they entailed were only possible because we had fun and did it not as a work, but as a way of building something together. Those days are long gone now, and research will probably never be as fun again.

It was not always great. Taking away the rose-colored glasses, I have to admit that it was sometimes terrible. The "ångest" (angst) generated from these projects was occasionally immense. Taking responsibility for your research, from the stage of project proposal all the way to presentation of results and all the issues that come with it is demanding for students yet to be established as researchers. You are on your own with your success and your failures. This "ångest" intermittently took its toll on our wellbeing and it made Per and Martin take a timeout from academia and focus on clinical work. And the ember of youth, which had kept us going in the face of hardships in the beginning of our careers, burned a little less brightly for each year that passed by.

I am privileged to have done the bulk of the work for this thesis together with two of my best friends and colleagues. In hindsight, one of the greatest challenges with the project has been to cope with the loneliness as they left.

A note on shared authorship

The standards for scientific authorship employed today could be considered as legacies from a time when Newton came to think about stuff while being hit by an apple in the head. Research today is often too extensive to be conducted by one person, too complex to be fully grasped by one brain and too multifaceted to have one single "lead author". How do you value different types of work? A great idea versus exhausting data gathering versus competence in statistical analysis; who deserves to be first and why should it matter that much anyway? As for the publications in this thesis, it would have been counterproductive to nominate a lead author. At stake when discussing these matters are the relations to your coworkers and friends. That is serious stuff; more serious than titles and academic prestige.

It may not be optimal for being fast-tracked in the science sector as shared authorship is "hard to evaluate" and it may be disturbing for old-school scientists valuing traditions highly. However, it was the only way to feel good and conserving our integrity while working in this team; something we regarded as our first priority in life. That is the reason we have chosen to solve the "credit issue" by shared authorship. No more, no less.

Thanks to the supervisors

I would like to express my deepest gratitude to professor Mikael Norman who accepted to supervise our Biafra-study and the vitamin D-study. We truly appreciate the confidence that you gave us and the scientific supervision you provided that made the projects possible.

Anna-Karin Edstedt Bonamy, has also been an invaluable support both scientifically and through frank and clear advices during the course of this thesis. Thank you.

Professor Sven Cnattingius supervised the last part of this thesis, the registry-based studies. I would like thank you for the support and the patience you had with me during the study, despite the numerous technical and methodological problems encountered on the way, and despite that I was always troubling you with my "krångel" (hustling/making things complicated by not settling with the standard procedures).

Thanks also to Fredrik Granath, the mastermind of statistics who outlined the analysis of the registry-based studies.

Anders Ekbom, the eminent boss of our department will I remain thankful to, for open-mindedly listening to my requests and arguments regarding my employment. I could not agree more with your words: "If you find yourself in an organization where you are not evaluated on results but on a lot of other things, leave that organization immediately". I will try to hold on to that.

Finally, I would like to thank Stefan Einhorn, my mentor, first for convincing me that obtaining a PhD is a good idea and later for the support and guidance during some periods of indecisiveness.

A global venture made possible by Google

This thesis would not have been possible to launch, or to even think about before the era of the internet. Without the internet, we would not have been able to rapidly gather information about previous research or to find relevant input instantly. We would not have been able to obtain contact information for our study participants or to have a calendar function for the massive scheduling of clinical investigations. And most of all, we would not have been able to swiftly establish contacts with colleagues in different parts of the world. Thank you Internet and Google.

I would like to thank our fellow researchers around the globe with whom we established new partnerships. Dr. Chima Charles, Professor Benjamin Ozumba and the students of Medix Frontiers who generously welcomed us to Nigeria and worked together with us during the field work. This project is truly a proof of what some arbitrary browsing on the web can lead to. I feel delighted to have lived and worked together with you and will always remain thankful for your generous assistance with the project.

Professor Darryl Eyles, Pauline Koh and Henry Simila from Queensland Brain Institute in Australia conducted the vitamin D-analyses in the dried blood spots. Thank

you for accepting our call for collaboration. I had the chance to meet with Darryl in person and was reminded of how inspiring and joyful science is.

Professor Gabrielle Doblhammer from the University of Rostock was generous in her advices when I got stuck in the registry study. Thank you for taking time although you didn't know me at all. I hope to meet you some day in the future.

Here in Sweden, Lene Sörensen and Ulrika von Döbeln at the PKU-bio bank have been fantastically kind when helping us with our work. Thank you!

And big thanks to the study participants in Nigeria and Sweden for kindly and patiently taking part in the clinical examinations.

Mr Kang Le and Ms Yuan Fang from Beijing, China generously provided some of the pictures used in this thesis.

We all love to complain on Karolinska, don't we?

During my time at Karolinska, I've been complaining a lot. On the bureaucracy, the lack of wifi, the conservative doctors, the paper-based communication systems, the outrageous food quality given the exorbitant prices in the hospital canteen, and the doctoral courses (some of them) with laxer passing requirements than in my high school. I'm happy to acknowledge however, that Karolinska has provided me with a sometimes surprising degree of freedom, trust and possibilities to explore the world. As the bureaucracy has been "hustleable", I have been able to take my own initiatives and assume responsibilities to run projects as I've wished. Karolinska has also furnished me with a strong brand, opening doors at various places on this planet. Although lacking a control scenario (what would have happened if I had gone to another med school and another institution for post-graduate studies?), I suspect that Karolinska has given me a great platform for my strivings. Of course, I am sincerely grateful for this.

At the end of the day…

So, with all these efforts made, all the opportunity costs and resources spent on this PhD-project and the science delivered… at the end of the day the unavoidable question remains to be asked… Did we create value? Did we contribute to the progress of science, human health and mankind or was this just one of those efforts made for the three magical letters after one's name and working hard for the sake of working hard? In the quest for growth of your publication list and the constant hunting for grants and new titles, we may not forget to consider this essential question.

As for the Biafra study, we concluded that pregnant women should not starve. Let me make clear that I think no one should ever starve. So, the value of this study, except from adding to the "body of evidence" regarding the importance of early life environment for future health is debatable, or as a Lancet reviewer (yes we did submit it to them (Hubris? Definitely)) put it pushing us over the edge to rejection (it was close however (indeed my respect for the journal would have been somewhat undermined should they have accepted it)):

" Whilst the authors are correct to indicate that this is the first study to link BP, glucose tolerance and obesity to malnutrition either in utero or during childhood in a sub-Saharian population, the data do not tell us anything new but merely confirm the observations of David Barker some 25 years ago, and those of others who have since studied the Dutch Famine."

Thanks for that… it hurts, but is probably true.

In the vitamin D-study, we actually examined a new hypothesis from a novel perspective. The study was designed based on the favorable circumstances for follow-up studies in Sweden using the PKU-biobank, the Swedish personal identification numbers and public information on the internet. To that we added the unique lab-methods developed in Australia by professor Eyles and colleagues. Although the study was limited by resource constraints, the findings were interesting and indicated that there could be more in this hypothesis to explore. This part of the thesis, therefore, I consider being a scientifically relevant initiative.

As for the registry-based studies, I fancied it being a fast and smooth project. This wasn't really the case. First there were the methodological issues, then the technical and once those had been resolved each analysis took about 25 hours to run, the longest 43 hours. And once the results were prepared I realized that they merely confirmed what had been reported elsewhere. Three months into the project I discovered a book called "the late life legacy of very early life" by professor Doblhammer. In this 200-pages book she crunches extensive data from all over the world to refute or confirm hypotheses regarding the month of birth effects that this thesis initially was aimed to investigate (latitude effects, causes of death etc). But she did not publish her results in articles searchable on Pubmed… After having elucidated every possible aspect of the topic, the book concludes that future research should focus on the mechanisms rather than replicating these findings. Could you please publish that conclusion in a searchable database or state it in on your blog or something before I spend half a PhD doing exactly that??!! Well, well… (However, as there could have been different results in Sweden compared to other countries, you could still argue that there was a decent rationale for the study)

This is what happens when you first create a project, and then try to justify it when the appropriate order should be the opposite (i.e. identify a need for knowledge, and then design a project to address it)! It also shows the disadvantages of designing your projects with your friends in the midst of an already swamped med-school life instead of relying on the established people with better overview of the research within the field of interest. I highly doubt that these studies will ever contribute to the improvement of people's health and I feel somewhat guilty for having spent my supervisors' time and resources on this, not to mention the questionable integrity as a science professional, doing

This thesis was supported by:

- The MD-PhD program, Karolinska Institutet
- Swedish International Development Cooperation Agency (SIDA)
- Frimurare Barnhusstiftelsen
- Karolinska University Hospital Huddinge
- Swedish Heart and Lung Foundation
- Sällskapet Barnavård
- Carl Erik Levins Stiftelse
- EU's 7th Framework Program. (no.259679)

The support is much appreciated. Thank you.

A special thanks also to the Tullus Family and the Carl Tullus Minnesfond for the support and encouragement.

work for the sake of publishing, rather than for a reasonable belief that it is of relevance. On the other hand, I may have thought otherwise, if the findings would have been more exciting.

It has to be noted, also, that this thesis, including clinical investigations in different settings, registry-studies with massive datasets and a lot of ambiguity, was pedagogically valuable for me as a student. And that is something that could further justify this thesis. The value of a PhD-project, thankfully, lies not only in the relevance of the science delivered but also in the education of the student. Thanks to great supervisors, colleagues and Karolinska, I got educated. I learnt some science, I learnt the basics of epidemiology and I learnt to survive in this part of society. Given that I can use this knowledge to perform scientifically useful and value-adding work of different types in the future, this thesis may well have served its purpose.

Thanks also to

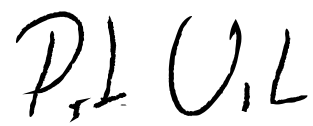
I am indebted (metaphorically and literally) to my parents, sister and grandfather for all the support and for the lifesaving injections to my bleeding economy during the harsh years when I made 12334 SEK (after-tax) a month. Without you I would have been out on the streets.

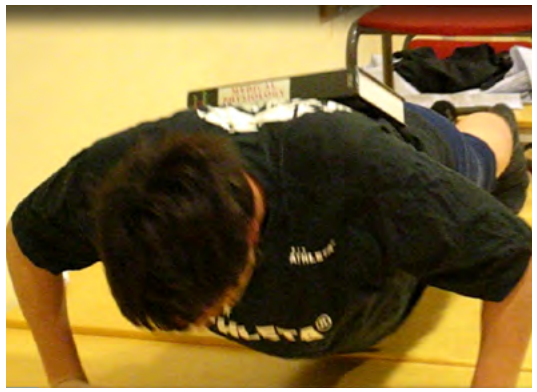
Thanks also to the Swedish Welfare System and the taxpayers for providing free education and to the Stockholm School of Economics for the quite extensive statistical courses that made me understand what I was doing in my research while patiently standing in line for the insufficiently sized doctoral courses at Karolinska. Furthermore, I would like to thank the Saito-family for kindly hosting me in Tokyo where much of the work for this thesis has been done, Makan Amini for being a vibrant source of inspiration and Mr Viedma for pushing my workaholic limits during the outrageous years in Lappis and for introducing me to a presentation philosophy that has leveraged my research a lot.

Lastly…

This thesis is the end of the end of a great and transformative period in life, including vast amounts of freedom, different types of activities in various countries, a lot of laughter, stress, tears, great people, broken hearts and youth. In life, we cannot hold on to things just because we like them at the moment, because we will change, the environment will change and people will change. We have to move on because if we stay for too long, we would merely replicate less good versions of our previous years. I will have to leave this place for a while, and I hope I see you soon again. From the bottom of my heart: Thank you.

Stockholm, August 2013





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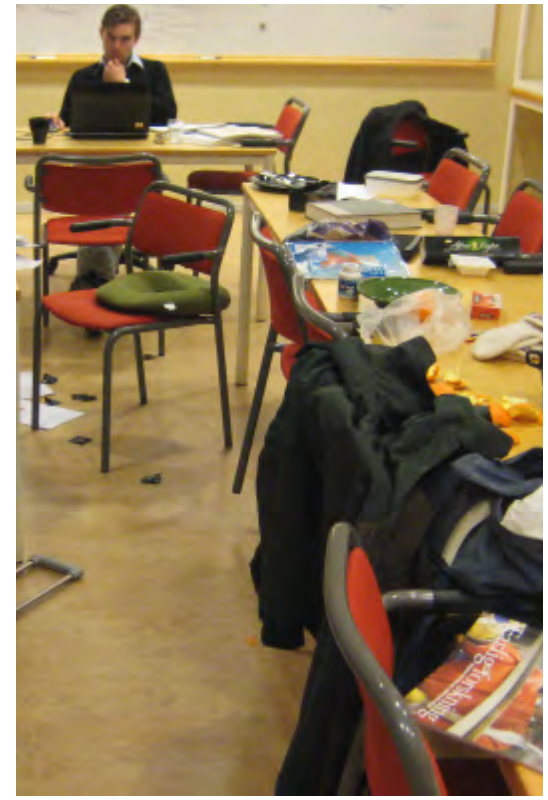
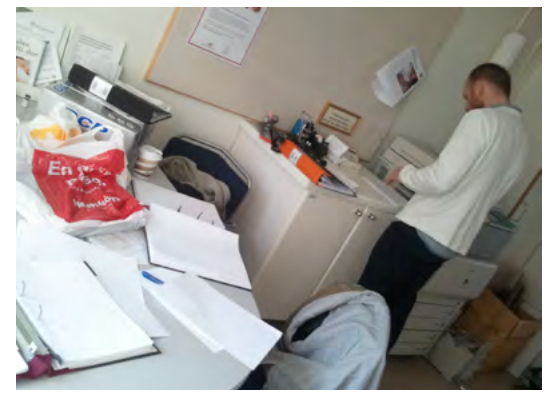




The New York Times

Nigeria: Those Born During Biafra Famine Are Susceptible to Obesity, Study Finds

By DONALD G. McNEIL Jr.
Published: November 1, 2010

Babies born during the brief but intense Biafra famine in Nigeria 40 years ago have grown up to be more susceptible to obesity and its attendant maladies than those born on either side of it, scientists have found.

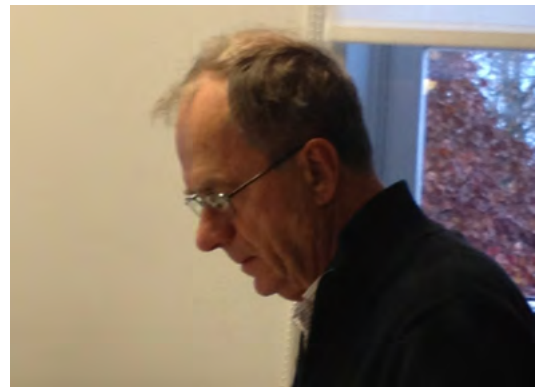
The researchers, from the Karolinska Institute in Sweden and the University of Nigeria Teaching Hospital in Enugu, said the finding added evidence to the argument that malnutrition in the womb causes greater susceptibility to such problems in later life.

Their study was published online last week by PLoS One.

↑ Martin Hult
↗ Per Tornhammar

↑ Anna-Karin Edstedt Bonamy
← Mikael Norman
↓ Sven Cnattingius

PEOPLE



↖ Benjamin Ozumba
↑ Darryl Eyles
← Stefan Einhorn

↑ Medix Frontiers
← Charles Chima

→ Mr Viedma